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Research Article

Morphological identification and *in vitro* management of *Alternaria alternata* causing fruit rot of persimmon

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ABSTRACT

Persimmon is one of the major fruit crops in Pakistan. Fruit rot of persimmon is a continuing threat to growers and is accountable for enormous economic loss. The investigations were done on persimmon fruit that were collected from local markets of Lahore. Diseased persimmon fruits were isolated and identified on the basis of cultural and morphological characteristics. On PDA, the cultivated isolates continuously produced dark brown to black colonies. Conidia had a small, conical beak with both transverse and longitudinal septa, and they were olivaceous to dark brown in color. Conidiophores were either branched or unbranched, short, septate, hyaline to olivaceous brown. Frequency of isolated fungi was calculated. The maximum percentage of *A. alternata* (61.00 %) was recorded followed by *C. gloeosporioides* (14.30 %) and *B. theobromae* (10.70 %). The least isolated pathogen was *R. stolonifer* (6.00 %) with a mean value of 5.00%. Evaluation of various plant extracts, such as (*Cascabela thevetia, Euphorbia milii, Moringa oleifera, Plumeria rubra, Quisqualis indica, Syzygium aromaticum*) was performed with Agar Well Diffusion Technique through CRD design The findings showed that with medicinal plants that were extracted with methanol and ethanol, *Moringa oleifera* and *Syzygium aromaticum* showed significant results at higher concentrations and showed maximum zone of inhibition while *Cascabela thevetia* was least effective against *S. sclerotiorum*.

Keywords: Persimmon, *Alternaria alternata*, Identification, Fruit rot, Agar well diffusion.

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INTRODUCTION

Persimmon (*Diospyros kaki* L.) is a fruit that belongs to the Ebenaceae family and is highly valued in East Asian nations. It is mostly grown in China, South Korea, and Japan (Izuchi et al., 2011). The popularity of persimmons has recently expanded beyond their traditional producing regions emerging as a promising crop in Brazil and other Mediterranean nations including Italy, Spain, and Portugal. In Europe, persimmon cultivation is restricted to areas around the Mediterranean Sea (Bubba *et al.,* 2009).

Approximately 3.03 million tons of persimmons are produced in China, followed by 400,000 tons in Spain, 300,000 tons in South Korea, 225,000 tons in Japan, and 182,000 tons in Brazil. One fifth of all fruits produced worldwide are persimmons, with a global production of around five million tons (FAO, 2017). In Pakistan, the fruit was introduced during 1940 in Mardan and know widely grown in various parts of the country. The fruit of the astringent commercial variety is available for harvesting in the start of October.

Persimmons have a brief ripening phase, thus fruit storage is required to preserve the quality of collected fruit and increase commercial periods. Fruit that is kept at low temperatures is susceptible to chilling harm, and several researchers have worked to manage this physiological problem (Besada et al., 2008).

Nevertheless, the increased frequency of fungal pathogen-induced postharvest illnesses, particularly Alternaria black spot (ABS), which is caused by *Alternaria alternata,* has hindered this extension of fruit postharvest life in recent seasons (Palou *et al.,* 2012).

Post-harvest deterioration poses a serious threat to persimmon productivity. Numerous variables, including significant injury, physiological changes, and pathological degradation, can contribute to post-harvest losses. The primary cause of postharvest losses in persimmons is fungus infections. Like many other crops, persimmons are susceptible to a variety of diseases and other factors that affect the branches, fruit, foliage, roots, and trunks of the trees (Cia et al., 2003). Fruit rot, stem end rot, soft brown rot, and anthracnose are a few post-harvest illnesses that lower fruit quality and cause significant losses. Latent infections such as anthracnose (caused by *Colletotrichum gloeosporioides*), *Alternaria* black spot (*Alternaria alternata*) and stem- end rot (caused by *Lasiodiplodia theobromae* or *Dothiorella dominicana* or *Botryosphaeria* spp.) are the predominant post-harvest diseases that cause severe post-harvest losses and affect fruit quality during the supply chain. (Kobile *et al.,* 2011). Spores, or inoculums, that come into contact in the post-harvest management of fruits procedures are the main source of the incidence of post-harvest deterioration. When the circumstances are right for germination and development, the spores are released into the air or by contact (Kwon *et al.,* 2004).

Due to incorrect harvesting, freezing injury, physical harm, fruit softening decay, spongy tissue, sap burn, lenticels discoloration, disease, and insect damage, persimmon post-harvest losses are substantial, especially in developing countries. Harvesting persimmon fruit at the right moment is essential for maximum sensory quality and longer shelf life since the fruit's quality and post-harvest life are dependent on its maturation stage (Goh *et al.,* 1991).

One of the main issues facing the persimmon sectors is postharvest management of persimmon fruits. The rising ecological consciousness of the public calls for the development of novel strategies to reduce fruit-borne diseases and enhance fruit quality and shelf life. Discouragement of pesticide use is also crucial to protect the environment and consumer health (Gerhardson, 2002). Medicinal herbs or plants are utilized increasingly as an environmentally friendly and safe substitute for synthetic fungicides due to their potent antifungal action against a wide variety of fungus. The current research assessed various portions of medicinal plants or herbs against *Alternaria alternata*. As a result, the objective was to distinguish the pathogen from persimmons exhibiting fruit rot symptoms and use methanolic and ethanolic medicinal plant extracts to combat *A. alternata*.

MATERIALS AND METHODS

Survey and Sample Collection:

The infected Persimmon fruit showing typical symptoms of fruit rot were collected from different fruit markets of Lahore and brought to Botany Laboratory of Institute of Molecular Biology and Biotechnology, The University of Lahore.

Isolation, Purification and Identification:

The isolations were performed using the methodology of (Ambreen et al., 2014). For this, the infected with healthy portions of the persimmon fruit peel were cut in to 1 cm pieces and surface sterilized in 70% ethanol for thirty seconds, the healthy portion was then washed with sterile water two times for 1 minute and dried on sterile filter paper. These pieces were placed on the petri dishes containing Potato Dextrose Agar (PDA) medium and after proper taging placed in the incubator for four to five days at 25 °C. To purify the isolated fungal pathogens, the hyphal tip approach was employed. Morphological traits such as spores color, shape and size were used to identify the species (Barnett and Hunter, 1998). Frequency percentage of isolated fungi was calculated by following formula:

$$\text{Frequency \%} = \frac{\text{Number of isolated fungi}}{\text{Total number of isolates}} \times 100$$

In vitro Antifungal Activity of Medicinal Plant Extracts:

Collection of plant parts

The medicinal plants (*Cascabela thevetia, Euphorbia milii, Moringa oleifera, Plumeria rubra, Quisqualis indica, Syzygium aromaticum*) were used in this study were taken from the local market.

Extraction

After collection of selected medicinal plant parts (Seeds, Leaves and Barks), first of all they were washed in tap water in order to remove dust particles. Afterwards, the plant pieces were allowed to air dry before being finely pulverized in a grinder. For every medicinal plant, 20 grams of pulverized plant material was weighed and applied individually to conical flasks containing 100% ethanol and methanol. Once at room temperature, the flasks were put on an orbital shaker for a duration of 12 hours. After that, the contaminants were taken out of the ethanolic and methanolic extracts

by filtering them through paper. A comparable clean solvent was used to extract the residues again, and the resulting extracts were combined. After being placed in a water bath at 50 degrees Celsius, the combined extracts were allowed to evaporate and become solvent-free. To prevent contamination and for future use, the evaporated extracts were refrigerated at 4ºC (Sultana *et al,* 2009).

Plant Extracts Antifungal Activity

The medicinal plants were for antifungal efficacy against *A. alternata* through agar well diffusion method (Perez *et al.,* 1990). Four concentrations (5 µg/ml, 15 µg/ml, 25 µg/ml and 50µg/ml) of medicinal plants were performed and mixed in PDA media properly. After that the prepared media were poured in the petri plates and placed for 20 mints for solidification. Purified fungal colonies were placed in the center of each plate and incubated at 28°C for 5 days and antifungal efficacy was evaluated.

Statistical Analysis

Using MSTAT-C computer program, the statistical analysis were performed on the data collected (Russel and Eisensmith, 1983). The overall significance of the data was tested using ANOVA method and the differences between the treatment means were compared using the LSD test (P≤ 0.l.).

RESULTS

Morphological Characterization of *Alternaria alternata*

Conidiophores produced by the fungus range in size from 25–60 x 3–3.5 µm and are pale brown to olive brown in color. Conidiophores, which consist of four to eight massive conidial chains, produce bushy heads. In addition to producing ellipsoidal, pale brown to light brownish spores that are smaller toward the tip, fungi also create tiny, single-celled secondary conidiophores. Their size varies from 20–63 x 9–18 µm, with a flat to verrucose surface. The size of their elder conidia is between 10–30 x 5–12 µm.

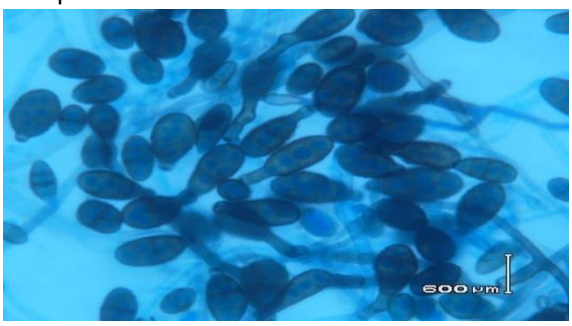

Figure 1: Conidia of *A. alternata*

Frequency of Isolated Fungal Pathogens

Five fungi including *Alternaria alternata, Aspergillus spp, Phomopsis mangiferae, Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. were isolated and identified based on the features of their morphology (Barnett, 1967). During isolations from diseased samples, maximum percentage of *A. alternata* (61.00 %) was recorded followed by *C. gloeosporioides* (14.30 %) and *B. theobromae* (10.70 %). The least isolated pathogen was *R. stolonifer* (6.00 %). (Table 1).

Table 1. Overall frequency of isolated fungal pathogens associated with diseases Persimmon *In vitro* antifungal assay.

Fungi isolated	Frequency (%)
Alternaria alternate	61.0 A
C. gloeosporioides	14.30 B
B. theobromae	10.70 C
Aspergillus spp.	8.00 D
Rhizopus stolonifera	6.00 E

Antifungal activity of six medicinal plants in ethanol were assessed against *Alternaria alternata.* The control plates were the ones devoid of plant extract. The inhibitory zone diameter was calculated in millimeters (mm) of ffour different concentrations includes 5 µg/ml, 15 µg/ml, 25 µg/ml, and 50 µg/ml. The effectiveness of every extract from medicinal plants against *Alternaria alternata* changed consistently after seven days. At a 50µg/ml concentration, *Moringa oleifera* produced statistically significant outcomes, with a maximum inhibitory value of zone 3.50 mm followed by *Syzygium*

aromaticum at 50µg/ml through DIZ value of zone was 3.26 mm. *Euphorbia milii* at the concentrations of 50µg/ml was least effective with 2.23 mm DIZ value. While in methanol, *Syzygium aromaticum* at the concentration of 50µg/ml exhibited maximum inhibitiory value of zone was 3.26 mm followed by *Moringa oliefera* at 50µg/ml with DIZ value of zone was 3.15 mm. *Plumeria rubra* at 50µg/ml was least effective with 2.56 mm DIZ zone value. (Table 2)

Table 2. Efficacy of different concentrations of ethanolic and methanolic extracts of medicinal plants against *A. alternata* after 7 days.

Treatments	Ethanolic extracts concentrations				Methanolic extracts concentrations			
	5µg/ml	15µg/ml	25µg/ml	50µg/ml	5µg/ml	15µg/ml	25µg/ml	50µg/ml
Cascabela thevetia	3.0abc	3.06abc	2.83abc	3.10ab	3.06ab	3.03ab	3.13ab	3.03abc
Euphorbia milii	2.83abc	2.53abc	2.6 abc	2.23c	3.1ab	3.06ab	3.03abc	2.9abcd
Moringa oleifera	2.53abc	3.13abc	2.53abc	3.5a	3.0abcd	2.9abcd	2.83abcd	3.15ab
Plumeria rubra	2.33bc	2.46bc	2.6abc	2.5abc	2.93abcd	2.6cd	2.8bcd	2.56d
Quisqualis indica	2.70abc	2.66abc	2.7abc	2.80abc	3.2ab	2.96abcd	3.0abcd	2.93abcd
Syzygium aromaticum	2.5abc	2.90abc	2.7abc	3.26abc	3.1ab	2.96abcd	3.1ab	3.26a
Control	0d	0d	0d	0d	0e	0e	0e	0e
Mean	2.49a	2.41a	2.28a	2.29a	2.65	2.51a	2.55a	2.5a
	LSD=1.01				LSD=0.46			

DISCUSSION

Alternaria alternata is a major postharvest pathogen that infects Persimmon fruit after harvest. The other fungi infect persimmon after harvest are *Colletotrichum gloeosporioides, Botryodiplodia theobromae, Phomopsis mangiferae* and *Aspergillus spp.* The infections are spreading because of the damp and humid weather. Conidia appear to be the main source of the infection.

This study found the morphology of colony demonstrated that single-spored colonies on PDA produced diagnostic characteristics that could be utilized to distinguish between A. alternata isolates that were typical of the species. According to earlier research by Andersen et al. (2001), differentiating of *A. alternata, A. infectoria A. longipes* and *A. gaisen*, and based on colony features was also made possible by incubation in darkness. There was a significant amount of carbohydrates in both the study's media and Andersen's, regardless of their differences. The presence of rich media encourages small-spored catenulate *Alternaria* spp to develop vegetatively while simultaneously reducing the number of sporulating hyphae (Simmons, 1992). In each of these investigations, the cultures were incubated in darkness, which further encourages vegetative development while limiting sporulation. Additionally, compared to colonies largely formed of highly sporulating hyphae, colonies composed mostly of vegetative hyphae display a greater variety in color and texture among small-spored catenulate *Alternaria* spp. Therefore, when colonies are developed on rich medium under darkness, the changes in their morphology are most noticeable.

The present study indicated that fruit rot of persimmon caused by *A. alternata*, is by a considerable margin the most important postharvest disease in Punjab Province.

All of medicinal plants were consistently found to be effective against the isolated fungal pathogen in minimizing mycelial development during the in vitro assessment process. Mycelial growth significantly decreased as plant extract concentration increased. *Moringa oleifera* and *Syzygium aromaticum* showed most inhibition activity against *Alternaria alternata* when extracted in ethanol and methanol at maximum concentration. The study findings were in line with the study conducted by Anthonia (2012), who found that at 30 ug/ml concentration of moringa exhibited the highest immunity to *Aspergillus flavus* and *Penicillium* spp. *Euphorbia milii, Plumeria rubra, Cascabela thevetia, Quisqualis indica* provided signifivcant results against *A. alternata* after extracted in methanol and ethanol at different concentrations. Same findings were reported by Maqbool *et al.,* (2010) studied the efficacy of four concentrations of *Euphorbia milii, Plumeria rubra* on conidial germination and mycelial growth of *A. alternata*. The results revealed that *Euphorbia milii,* exhibited significant findings against *A. alternata* extracted in methanol at maximum concentration. Necha *et al.,* (2008) investigated the efficacy of nine medicinal plant extracts on mycelial growth inhibition and conidial germination of *A. alternata.* The results revealed that *Syzygium aromaticum* was excellent in inhibition of *A. alternata* isolated from different fruits at all tested concentrations. Sukatta *et al.,* (2008) assessed the significant growth inhibition efficacy of *Syzygium aromaticum* against six phytopathogenic fungi caused post-harvest perishes; *Alternaria alternate, Aspergillus niger, Rhizopus stolonifer*, *Phomopsis viticola, Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides*.

CONCLUSION

It was concluded from current research that *Alternaria alternata* was most devastating fungal pathogen of persimmon in district Lahore. Furthermore, it was observed that different medicinal plant extracts (*Moringa oleifera* and *Syzygium aromaticum*) can be utilized as an substitute to synthetic fungicides to control postharvest fruit rot of persimmon.

COMPETING OF INTEREST

The authors declare no competing interests.

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