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CHARACTERIZATION OF *CURVULARIA LUNATA;* FOLIAR FUNGAL PATHOGEN OF ORNAMENTAL PALMS

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ABSTRACT

Agriculture is the backbone of any economy and an essential field of study considered as a challenge for many researchers today. Fungal diseases are a constant threat to agricultural commodities resulting huge decline in quality and quantity. The early detection and classification of fungal plant diseases are crucial for preventing growing diseases and hence yield reduction. In the present study, leaf spot on ornamental palm, caused by *Curvularia lunata*, was identified as a major fungal pathogen declining the quality of palm trees. The fungus was isolated from lesions on leaves, and pathogenicity was confirmed. Pathogenicity assay confirmed that *C. lunata* is a causal agent of leaf spot of ornamental palms. The fungus was identified based on morphological and microscopic characteristics. One of the characteristics of palm disease is that the disease symptoms mostly appear as spotted on the leaves at its nursery stage. So, the need of time is to develop a novel and authentic disease detection system that may detect and separate the infected palm trees earlier to prevent the reduction of palm yields.

Keywords; *Curvularia lunata*, Ornamental Palm Tree, Pathogenicity Assay.

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INTRODUCTION

Palms include a natural and characteristic, yet remarkably tropical plant comprising about 2500 species in 184 genera and is best and famous in, the Central South America

western Pacific, Australia tropical Asia, and Madagascar (Dransfield et al., 2008). Numerous palms hold great worldwide commercial significance. The date palm and coconut palm are considered two of the world's ten most significant agronomic crops (Janick & Paull, 2008). Palms are good source of food and oil, fiber for coir, string, bins, caps and carpets; cane used in furniture manufacture, tannin, timber, straw for roofing material, wine and other drinks (Howard et al., 2001). Currently there is bigger command for date palm fruits in all over the world. To full fill this mandate numerous production techniques have been exploited among them micro propagation which has been used in Iraq and various other countries for large-scale multiplication of date palm. Palm a tropical tree crop, is important for the industrial production of vegetable oil with Indonesia and Malaysia and makes up the highest plantation area in Southeast Asia (Carter et al., 2007).Quality of palm tree is mainly affected by various fungal rots. Pink rot (*Nalanthamala vermoesenii*), false smut (*Graphiola phoenicis*) and black rot (*Ceratocystis paradoxa*) are the most destructive fungal diseases of palm, while *Botrytis phaeriaceae*, Pestalotiopsis spp., and Phomopsis spp., are found to be the common diseases (Cabrera et al., 1990). Many of the leaf spots and leaf blights of palm are initiated by fungal pathogens. Generally, the symptoms appearance alike which fungus is causing the spot or blight. Palm leaflets are normally formed in cross section with the middle vein at the apex of the v shape (Broschat, 2016). Armengol et al., (2002) reported that Some species of Phytophthora cause main diseases of palms all over the world with bud (heart) rot the very common and disturbing disease. A lethal bud rot of numerous species of palms is caused by Phytophthora palmivora (Elliott et al., 2004).

Curvularia leaf spot disease caused by *Curvularia lunata* is a unique fungus in appearance and life cycle, but it is broadly dispersed all over the date palm-growing domain. Although several palm species have been recognized as hosts of this fungus the disease is widespread worldwide (Elliott et al., 2010). To evaluate the cause of decline in palm quality the present study was focused on identification and characterization offoliar fungal rot of ornamental palm trees.

MATERIAL AND METHODS

The current study was designed to determine the isolation and preservation of important foliar fungal pathogens along with their morphological and cultural studies. An extensive survey was conducted of district Islamabad Pakistan (33.6841° N, 73.0480° E) during year 2021 to identify foliar fungal rot of ornamental palm trees. Infected leaves were washed thrice with distilled water and blotted dry. Diseased portions were excised into $5mm^3$ segments including diseased as well as healthy portion. These segments were surface disinfected with one percent sodium hypochlorite (NaOH) for about 1-2mins and washed twice with sterilized distilled water. Disinfected samples were blotted dry using sterilized filter papers and were placed on PDA media petri plates and incubated at 25ºC±2 for a week. Isolated fungal pathogen was purified by single spore method and hyphal tip method. After purification fungi was identified under microscope based on colony color, colony texture, mycelia appearance, hyphal septation. Spore size and shape was observed under 100X lens of Nikon YS100 microscope using fungal taxonomic identification keys.

Pathogenicity Assay was conducted on ornamental palms for the confirmation of Koch's postulates. The inoculum of each strain was prepared by flooding the agar surface with 10 mL of sterile distilled water (SDW) and scraping with a spatula. The resulting spore suspension was filtered through four layers of cheesecloth and the filtrate was diluted with sterile distilled water whereas the conidial concentration was adjusted to 10^6 conidia mL^{-1} using a haemocytometer. One-year healthy ornamental palm leaves were sterilized with 0.5% sodium hypochlorite (NAOH) solution and placed in sterilized boxes. Further inoculation was done by spraying inoculum on with approximately 50 mL of spore suspension. Another healthy ornamental palm leaves inoculated for controls was sprayed with sterile distilled water and incubated for 5- 10 days till the appearance of symptoms.

RESULTS AND DISCUSSION

Disease incidence of four abundantly found palm spp., was recorded in Islamabad Pakistan. Maximum disease incidence was recorded 74.21% of Italian palm and minimum disease incidence recorded was 43.11% of table palm (Figure 1). A total of

23 isolates were subjected to morphological studies. The pathogen identified on morphological and microscopic features was *Curvularia lunata* initiating black leaf spot of bamboo palm. Colony color varied from light brown to brownish black and light brown color. Among 23 isolates, CURL3 was observed with maximum colony diameter and CURL2 was recorded showing minimum colony diameter 66 mm. Colony margin color varied from blackish brown to dark black with somewhat straw color appearance. Growth pattern of colonies was recorded suede-downy, irregular to appressed having fluffy growth. Underside of petri dish was observed blackish-brown. Conidia were boat shaped showing olive green to dark brown (Figure 2). Spore shape of isolates was straight to curved and maximum spore length 38.14±4.34 µm was observed in CURL3 whereas minimum spore length 22.11±2.13 µm was observed in CURL5 (Table 1; Figure 3). During pathogenicity assay 06 isolates of *Curvularia lunata* were found to be highly pathogenic. Fungal isolates were preserved in 15% glycerol solution and were placed in -20 C for long term usage.

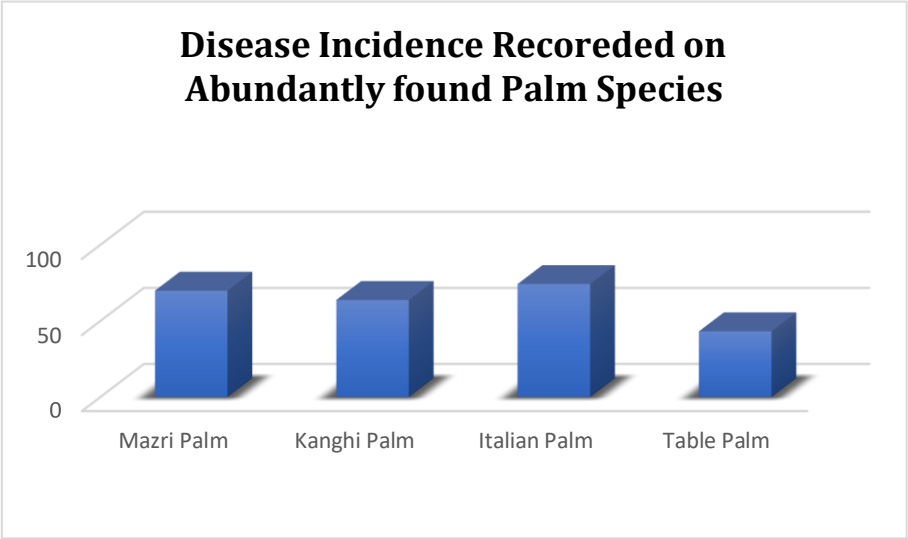

Figure 1 Disease Incidence on Abundantly found Palm Species

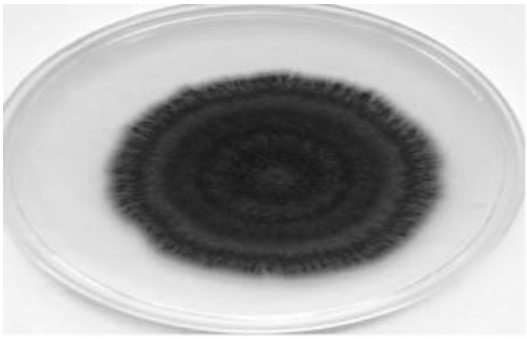

Figure 2 *Curvularia lunata* pure culture on PDA media

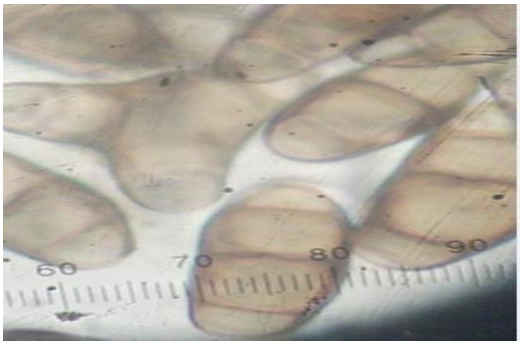

Figure 3 Spores observed under Nikon Microscope

Table 1: Cultural characterization of *Curvularia lanata*

Isolate ID			**Pattern**	**Diameter**	**Colony Co (Length)**
CURL1	Light black-brown	Boat Shaped	Suede-downy	68 mm	31.27±3.14 µm
CURL2	Blackish-brown	Enlarged	Irregular appressed	66 mm	33.41±4.11 µm
CURL3	Black	Boat Shaped	Fan-like appearance	72 mm	38.14±4.34 µm
CURL4	Light brown	Boat Shaped	Smooth	67 mm	29.25±3.21 µm
CURL5	Dark Black	Boat Shaped	Suede-downy	69 mm	22.11±2.13 µm
CURL6	Black	Boat Shaped	Suede-downy	71 mm	29.91±2.17 µm

In another study reported by Manamgoda et al., (2012) morphological features of fungal pathogen causing leaf spot of ornamental palm were evaluated where colonies were observed whitish to greyish black mycelial growth after 8 days of incubation at 26 °C, moreover colonies were blackish, conidiophores were septate with dark brown scars, unbranched, and fexuose at apical region, conidia were dark brown, boat shaped with rounded tips, smooth walled, in sympodial order and 25–33×12–14 µm size (Manamgoda et al. 2012). It is pertinent to mention that *Penicillium* spp., *Fusarium* spp., *Curvularia* spp., *Aspergillus* spp., and *Alternaria* spp., are important pathogens of ornamental palms causing significant yield losses to date-palm (Bokhary, 2010; Khudhair et al., 2015; Suwannarach et al., 2015; Polizzi and Vitale, 2003). In addition, *Curvularia lunata*, *C. maculans* and *Helminthosporium halodes* have also been reported as oil palm leaf spot pathogens (National Research Council 1993). Furthermore, leaf spot on ornamental and oil palms is initiated by *Curvularia lunata* in Pakistan and worldwide (Farr and Rossman 2020).

CONFLICT OF INTEREST

No conflict of interest declared by authors.

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