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Identification and measurement of spore size of *Oidium erysiphoides* f. sp. *ziziphi* on *Ziziphus mauritiana* Lam. found in University Campus Kota Rajasthan India

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Abstract

Ber (*Ziziphus mauritiana* Lam.) is an ancient and poor man's fruit crop grown in semi arid and arid regions of India and other few countries. In India, Z. *mauritiana* trees are a host for the lac insects, Kerria Lacca, which are found on the leaves and make an orange-red resinous substance widely used to make varnish (Sajeewa SN *et al*. 2011). Disease specimens were collected throughout the year from different parts of University campus Kota, Rajasthan India, where the plant was seen and the associated pathogens were isolated following standard laboratory techniques. Detailed microscopic studies were carried out in laboratory. The causal organism found was powdery mildew incited by Oidium erysiphoides f. sp. *ziziphi* Yan & Wang (*Microsphaera alphitoides* f. sp. *ziziphi* Griffon & Maublanc). It is a major disease in Ber producing regions of India, causing great loss in productivity and quality of fruits (Jamadar MM, Balikai RA and Sataraddi AR 2009, Lim *et al*. August 2003). This fungi is obligate parasite that require living hosts in order to complete their life cycles, so they readily infect healthy, vigorous plants. Therefore the present study was conducted to understand the knowledge about the causal organism found in University Campus Kota Rajasthan India and to further develop efficient management strategies for control of this disease.

Keywords: Ber, Causal organism, Disease symptom, Media

Introduction

Ber (*Ziziphus mauritiana* Lam.) is distributed worldwide, including the Indian sub-continent, Southeast Asia, Australia, China, Africa, the Mediterranean region and the American center but its cultivation is confined over drier parts of the globe and commercial cultivation occurs in India. It is an example of an extremely drought-hardy species and is a dominant component of the natural vegetation of the Indo-Pak deserts. (Sarolia DK *et al*. 2024) [17]. According to De Condolle (1886), the center of origin of ber is Central Asia, where it is found under varying climatic conditions. It is grown in India traditionally from ancient times, where it has been in use for almost 4000 years. Ber, Indian jujube, Indian plum, Chinese date or Chinee apple is a hardy fruit crop well-suited for the arid conditions of Rajasthan. Fruit formation occurs between January and March. It is one of the most important fruit crops of tropical and subtropical region in India. Ber is known as Poor Man's Fruit or King of Arid Fruits. Ber plant also suffers from various diseases (Riker AJ and Riker RS, 1936; Quan-Yu Jie, 2000) [15, 14]. In the present study pathogen was identified and isolated from infected disease samples collected from different sites of Kota University campus Kota Rajasthan India.

Materials and Methods

About the plant

Ziziphus mauritiana is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 50 cm or more in diameter; spreading crown; stipular spines and many drooping branches. Bark dark grey or dull black, irregularly fissured. It belongs to the family Rhamnaceae. Leaves variable, alternate, in 2 rows, oblong-elliptic, 2.5-6 x 1.5-5 cm, with tip rounded or slightly notched base; finely wavy-toothed on edges, shiny green and hairless above; dense, whitish, soft hairs underneath. Inflorescence axillary cymes, 1-2 cm long, with 7-20 flowers; peduncles 2-3 mm long; flowers 2-3 mm across, greenish-yellow, faintly fragrant; pedicels 3-8 mm long; calyx with 5 deltoid lobes, hairy outside, glabrous within; petals 5, subspathulate, concave, reflexed.

Fruit is a drupe, globose to ovoid, up to 6 x 4 cm. Ber is a perennial, tropical fruit crop used in dyeing silk and trees used for rearing of lac insect and other medicinal purposes.

Experimental site

All the laboratory experiments were carried out at Department of Botany, University of Kota Rajasthan India

Site selection for collection of disease samples:

The samples of diseases were collected from different sites of university campus Kota Rajasthan India.

Culture media

Growth characters of powdery mildew was studied on solid media-PDA (potato, dextrose, agar) and plain agar extract (Sharma G and Pandey RR, 2010; Maurya AK, 2019) [18, 12]. All the media was sterilized at 1.1kg/cm pressure 121 for 15min. To carry out the study 20ml of each of medium is poured on 90mm petriplates. Such plates were inoculated with 5mm disc cut and incubated at 28 temperature.

Symptoms

Visual examination

Visual observation were made for identification of different symptoms on leaves and fruits in Ziziphus. Symptoms often first appear on the upper leaf surface, but can also develop on lower leaf surfaces. diagnosis of powdery mildew is not difficult, symptoms often escape early detection if plants are not periodically monitored since symptoms can first develop on lower or middle leaves. Masses of white powdery spores on surface of leaves and fruits were seen (Fig 1). The developing young leaves show a white powdery mass causing them to shrink and defoliate. Small, white powdery growth appear on the young fruits which later enlarge and coalesce and final turn brown to dark brown. In severe cases, the whole fruit surface was covered with the powdery mass. Affected young fruits drop off prematurely or become corky, cracked, mis-shapen and underdeveloped. Matured fruits turn rusty.

Fig 1: Infected plant of *Ziziphus mauritiana*

Microscopic examination

Diseased samples exhibiting symptoms of powdery mildew were brought to laboratory and washed in tap water.

Isolation of causal organisms: For isolation of causal organisms from the collected disease samples, small triangular or square shaped sections were cut containing infected portion were cut with scissor, then the square sections were washed in running tap water properly and surface sterilized in 70% ethyl alcohol or 0.1% mercuric chloride for 15 seconds under laminar air flow to avoid surface contamination (Brown W 1924, Devi KS *et al*. 2018) [1, 3]. These sections/pieces were transferred aseptically to the petriplates containing PDA and incubated at 28±2 °C. After 5-7 days of incubation a white or grayish white mycelial growth appeared from the surface of the section.

Microscopic observation: Diseased samples were observed under microscope at different power of 5X, 10X, 40X and photgraphs of spores were taken. spores were observed on glass slides under microscope by staining with lactophenol stain. For measurements of fungal spore at first standardization of ocular micrometer with stage micrometer was done. Each fungal isolate was taken into clean slides and then one drop of lactophenol was added on that. After teasing coverslips were used to cover the fungal culture and kept under microscope. Ocular micrometer was inserted inside the eye piece and length and breadth of the fungal conidia was measured.

Pathogenicity test

Healthy Ber were surface sterilized with 0.1% mercuric chloride for 2-3 minutes' followed by 3times washing with sterile distilled water. Ber fruits were then inoculated by spraying conidial suspension with the help of atomizer which was prepared by scraping and blending of 7day old culture grown on PDA media (Ikechi-Nwogu CG and Elenwo EN, 2012) [6]. Fruits were then kept in polythene bags incubated at 25 °C for 10 days. After 15–20 days pathogens produced similar symptoms like before. Pathogens were reisolated from fruits which showed symptoms after incubation. Pathogenicity test were done based on Koch postulate (Jorgensen JH *et al*. 2015) [8].

Observation and results

The detailed macroscopic and microscopic features of fungal colonies developed from leaves and fruits of Ber was identified as *Oidium zizyphi* or *Oidium erysiphoides* f. sp. *ziziphi*. Oidium erysiphoides-the fungal pathogen is an ecto-parasitic in nature. The pathogen colonizes on the epidermal layer of the host tissue. Microscopic examination of fungal isolates in lactophenol stain revealed typical conidiophores, septate hyphae, bearing hyaline conidia at the tip. Conidiophores are upright and had swollen or bulbous dark colored basal cell at the base of conidiophores which is the major characteristic feature of this powdery mildew pathogen Mycelium was hyaline, branched, septate thick walled and gave rise to a single conidiophore. Oidium erysiphoides forms hyphae, which are thread-like structures that grow on the surface of the host plant. It also produces asexual spores called conidia, which are white and arranged in chains or pseudichains. sexual fruiting bodies called cleistothecia (or chasmothecia), which are small, spherical, and initially white, later turning yellow-brown. Conidia

were hyaline, oblong-elliptical, measured 30 to 48 × 13 to 18 µm (Fig 2). Initially, the fungal colonies appeared as white and then with time turning to light brown. The colony diameter was ranged from 60-70mm (PDA), surrounded by white circle. The colony texture was floccose, with white mycelium sparse on the colony.

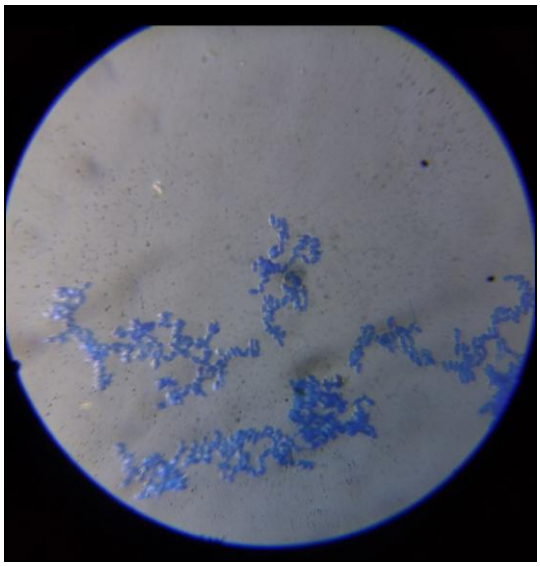

Fig 2: Conidia of *Oidium erysiphoides*

Discussion and conclusion

The Ber is one of the important minor fruit crop prone to be attacked by numerous fungal and algal diseases. (Gupta PC and Madaan RL, 1977; Gupta Dolly and Razdan VK, 2010) [5, 4]. The symptoms of these diseases were found to be similar with the outlines of disease symptoms exerted by different scientists (Choi *et al*, 1999 Gupta Dolly and, Razdan VK, 2010; Yuan Gao Qing *et al*. 2009) [2, 4, 19]. Pathogens of fungal diseases was identified based on their etiology under microscopic study and previous workers' references as well as pathogenicity test (Kapur SP, Chema SP and Singh MP, 1975; Kumar A, Bhansali RR and Arya HC, 1978; Jamadar MM, Balikai RA, and Sataraddi AR, 2009; Quan-Yu Jie, 2000) [9, 10, 7, 14]. Hence from this study the basic idea was obtained about characteristic symptoms of diseases of Ber in University Campus Kota Rajasthan India along with their infecting agent and morphological structure and ultimately their suitable media for easily isolation.

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