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Pathogenicity test of major fungal pathogens in vanilla (*Vanilla planifolia* Andrews)

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Abstract

Vanilla (*Vanilla planifolia* Andrews) is an economically important spice crop affected by several fungal diseases that significantly reduce yield and quality. The present investigation was undertaken to isolate, identify, and prove the pathogenicity of major fungal pathogens associated with vanilla under Konkan conditions. Diseased samples of leaves, stems, roots, and pods were collected and subjected to tissue isolation on Potato Dextrose Agar (PDA). The isolated fungi were purified, mass multiplied, and tested for pathogenicity on healthy vanilla plants and pods following Koch's postulates. Five major fungal pathogens, namely *Colletotrichum gloeosporioides* Penz., *Fusarium oxysporum* F. sp. *vanillae* Schlecht, *Rhizoctonia bataticola* (Taub.) Butler, *Sclerotium rolfsii* Sacc and *Aspergillus niger* van Tieghem, were isolated and confirmed. Pathogenicity tests revealed characteristic symptoms such as stem rot, leaf spot, basal rot, wilting, and pod rot. Reisolation of pathogens confirmed their causal role. The study highlights the importance of these fungal pathogens in vanilla cultivation and provides a basis for developing effective disease management strategies.

Keywords: Koch's postulates, vanilla, fungal pathogens, pathogenicity test, stem rot, pod rot etc.

1. Introduction

Vanilla (*Vanilla planifolia* Andrews), a perennial climbing orchid belonging to the family Orchidaceae, is cultivated commercially for its cured beans rich in vanillin, which imparts characteristic aroma and flavour. Vanilla is widely used in food, beverage, pharmaceutical, perfumery, and tobacco industries and is popularly known as the "Prince of Spices". In India, vanilla cultivation has expanded to states such as Karnataka, Kerala, Maharashtra, Andhra Pradesh, and parts of the Konkan region due to its high economic returns.

Vanilla thrives under warm and humid climatic conditions with well-distributed rainfall and moderate shade. However, the crop is highly susceptible to a range of fungal and viral diseases that cause considerable yield losses. Important fungal diseases include stem rot, root rot, basal rot, leaf spot, anthracnose, bean rot, and shoot tip rot. With the intensification of vanilla cultivation, disease incidence has increased, necessitating systematic studies on causal organisms.

The present investigation was therefore carried out to isolate major fungal pathogens associated with vanilla, identify them based on morphological characteristics, and confirm their pathogenicity through Koch's postulates.

2. Materials and Methods

The present investigation was undertaken to isolate, identify, and prove the pathogenicity of major fungal pathogens associated with vanilla under Konkan conditions.

Isolation of fungal pathogens

Diseased samples of vanilla leaves, stems, roots, and pods showing typical symptoms were collected. Small infected tissue bits were surface sterilized using 0.1% mercuric chloride for two minutes, followed by three washings with sterile distilled water. The tissue bits were placed on Potato Dextrose Agar (PDA) plates and incubated at 28 ± 1 °C for seven days. Fungal colonies emerging from the tissues were examined microscopically and sub-cultured.

Purification and Maintenance of cultures

Pure cultures were obtained using the single hyphal tip method. The purified isolates were maintained on PDA slants under aseptic conditions and stored at optimum temperature for further studies.

Mass Multiplication of Inoculum

Each fungal isolate was mass multiplied on Potato Dextrose Broth (PDB). Sterilized PDB in 500 ml conical flasks was inoculated with 5 mm mycelial discs from 7-day-old cultures and incubated at room temperature for 10 days.

Pathogenicity Test

One-month-old healthy vanilla vines grown in pots were used for pathogenicity tests. The mycelial mat and spores from PDB cultures were blended with sterile water to obtain a uniform inoculum. The inoculum was applied to the soil near the collar region or on slightly injured plant parts, depending on the pathogen. Control plants were maintained without inoculation. Inoculated plants were covered with polythene bags to maintain high humidity and observed regularly for symptom development.

Re-isolation and Identification

Pathogens were reisolated from artificially infected plants showing typical symptoms and cultured on PDA to confirm Koch's postulates. Identification of pathogens was carried out based on cultural and morphological characteristics and confirmed at NIKU Bio-Research Laboratory, Pune.

3. Results and Discussion

Isolation and Identification of Pathogens

Five fungal pathogens were consistently isolated from diseased vanilla samples. These included *Colletotrichum gloeosporioides*, *Fusarium oxysporum* F. sp. *vanillae*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Aspergillus Niger*. Identification was based on colony morphology, mycelial characteristics, and spore structures, which were in agreement with standard descriptions reported in the literature.

3.1 Pathogenicity Tests

Colletotrichum gloeosporioides

Inoculated vanilla plants developed water-soaked lesions at the collar region 18 days after inoculation, followed by chlorosis, stem rot, and shoot tip rotting. Complete wilting occurred within 45 days. Similar symptoms have been reported earlier for anthracnose and stem rot of vanilla by Amaresh *et al.* (2005) [2].

Also similar results for *Colletotrichum* sp. were obtained on inoculation by Thakare and Patil (1995) [6], Mertely and Legard (2004) [4] when associated with chrysanthemum and strawberry, respectively.

Fusarium oxysporum F. sp. *vanillae*

Symptoms appeared 11 days after inoculation as water-soaked lesions near the soil line, progressing rapidly to stem rot and die-back of the shoot tip. Complete wilting occurred within 20 days, confirming its aggressive pathogenic nature. Alconero (1968) [1], also proved the pathogenicity of species of *Fusarium* and *Rhizoctonia* on vanilla.

Rhizoctonia bataticola

Water-soaked lesions appeared within six days of

inoculation, followed by basal stem discoloration, shredding, and complete wilting within 30 days. The symptoms closely resembled natural infection observed in the field. Proved the pathogenicity of *Rhizoctonia bataticola* on cowpea. The inoculated plants showed typical symptoms of leaf blight after 8-10 days of inoculation. Patil (2003) [6] proved the pathogenicity of *R. bataticola* causing stem and root rot of sunflower in field condition and also in pot experiment.

Sclerotium rolfsii

Infected plants showed collar rot symptoms 12 days after inoculation, with white radiating mycelia and sclerotial formation on soil and stem surfaces, leading to girdling and plant death. Shredding of stem and roots was observed only in infection of *Rhizoctonia* sp. In support of these findings, Ashoka *et al.* (2006) [3] similarly reported for root rot of vanilla caused by *Rhizoctonia bataticola* (Taub.) Butler. Thomas *et al.* (2002) [9] also proved pathogenicity of *S. rolfsii* causing bean and stem rot of vanilla.

Aspergillus Niger

Inoculation of fresh green vanilla pods resulted in yellowing, distal-end rotting, wrinkling, and development of foul odour. Reisolated cultures were identical to the original isolate, confirming pathogenicity. The successful reproduction of disease symptoms and reisolation of pathogens fulfilled Koch's postulates, establishing the causal role of these fungi in major vanilla diseases. Reddy *et al.* (1991) [7] proved that groundnut seed germination was affected by pathogens like *A. Niger*, *A. terreus*, *A. Niger*. Suryawanshi (2005) [8] proved the pathogenicity of *Aspergillus Niger* on vanilla pods.

4. Conclusion

The study confirmed that *Colletotrichum gloeosporioides*, *Fusarium oxysporum* F. sp. *vanillae*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Aspergillus Niger* are major fungal pathogens associated with vanilla. Their pathogenic potential was conclusively demonstrated through artificial inoculation and reisolation. Understanding the pathogenic behaviour of these fungi is essential for developing integrated disease management strategies to minimize yield losses in vanilla cultivation.

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