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Morphological study of gummy stem blight pathogen of watermelon (*Citrullus lanatus*) in Andhra Pradesh

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Abstract

Gummy stem blight (GSB) is one of the most destructive fungal diseases of watermelon (*Citrullus lanatus*), causing severe yield and quality losses, particularly under warm and humid environmental conditions. The disease affects plants at all growth stages and is characterized by leaf blight, stem cankers with gummy exudation, vine collapse, and fruit rot, thereby posing a serious threat to watermelon cultivation. The disease is associated with *Stagonosporopsis cucurbitacearum*. The present investigation was undertaken to isolate and characterize the causal organism of gummy stem blight of watermelon based on cultural, morphological and pathogenic characteristics. Infected watermelon plant tissues exhibiting typical gummy stem blight symptoms were collected from affected fields. The pathogen was isolated from diseased tissues and cultured on potato dextrose agar (PDA) under laboratory conditions. Observations on colony morphology were recorded. The fungal cultures initially produced white to pale grey colonies, which gradually turned dark grey to black with age due to the formation of pycnidia. The hyphae were septate, profusely branched and hyaline to light brown in colour. The study highlights the preliminary identification of *Stagonosporopsis cucurbitacearum* as the causal agent of gummy stem blight disease based on morphological characterization. It provides a sound basis for further studies.

Keywords: Gummy stem blight, watermelon, *Stagonosporopsis cucurbitacearum*

Introduction

Watermelon (*Citrullus lanatus*) is a commercially important cucurbit crop grown widely across the world for its high nutritional value and market demand. It belongs to the family Cucurbitaceae and has a chromosome number of $2n = 2x = 22$. The crop is believed to have originated in the Kalahari Desert of Africa and was later domesticated in regions such as Egypt and India (Paris, 2015; Renner *et al.*, 2017) ^[16, 18]. In India, watermelon is cultivated over an area of about 101 thousand hectares with a total production of approximately 24.80 lakh tonnes. Major watermelon-producing states include Punjab, Haryana, Karnataka, Assam, Madhya Pradesh, Andhra Pradesh, Maharashtra, Telangana, West Bengal, Odisha, Himachal Pradesh, Uttar Pradesh, and Tamil Nadu (Anonymous, 2017) ^[2]. The crop is commonly referred to by different local names such as tarbuj, tarmuj, kalinda, kallangadi, and kalindi in various parts of the country (Li *et al.*, 2015) ^[12]. Despite its economic importance, watermelon production is constrained by several diseases, among which gummy stem blight (GSB) is regarded as one of the most destructive. The disease affects multiple plant parts, including leaves, stems, vines, and fruits, leading to leaf blight, stem cankers accompanied by gummy exudation, vine collapse and fruit rot (Al-Jubouri and Hussain 2020) ^[1]. Gummy stem blight of watermelon is caused by the fungus *Stagonosporopsis cucurbitacearum*, which represents the anamorphic stage of the pathogen. In India, the disease was first reported on chow-chow in Mysore (Sohi and Prakash, 1972) ^[20] and was later recorded on other cucurbit crops such as cucumber, bitter gourd, ash gourd (Pandey and Pandey, 2003) ^[15], muskmelon (Sudisha *et al.*, 2003) ^[21], and ridge gourd (Bhat *et al.*, 2018). In the present investigation, the causal organism of gummy stem blight was isolated from infected watermelon tissues using the standard tissue isolation technique. The identity of the pathogen was confirmed based on its morphological, cultural, and mycelial characteristics, following the descriptions provided by Li *et al.* (2015) ^[12], Moumni *et al.*

(2019) ^[14], and Mahapatra *et al.* (2020) ^[13], Cui *et al.* (2024) ^[6]. A severe incidence of gummy stem blight was observed in the Annamaya district of Andhra Pradesh. Therefore, the present study was undertaken to characterize morphologically the pathogen associated with gummy stem blight of watermelon.

Materials and Methods

Collection of Diseased Samples

Watermelon plants showing typical gummy stem blight symptoms such as brown to black leaf lesions, stem cankers with amber-colored gummy exudation, vine wilting, and fruit rot were collected from affected fields located in Anantharajupeta, Andhra Pradesh. Samples were brought to the laboratory in sterile polythene bags for further analysis.

Isolation of the Pathogen

Small tissue bits (5-10 mm) were cut from the advancing margins of infected leaf, stem, vine, and fruit tissues. The tissue bits were surface sterilized using 1 per cent sodium hypochlorite solution for 30-60 seconds, followed by rinsing three times in sterile distilled water to remove traces of the sterilant. The sterilized tissue pieces were blot-dried using sterile filter paper and aseptically transferred onto potato dextrose agar (PDA) plates. The inoculated plates were incubated at 25 ± 2 °C for 7-10 days under laboratory conditions. Fungal growth emerging from the tissue bits was sub-cultured onto fresh PDA plates. Pure cultures were obtained using the hyphal tip method and maintained on PDA slants at 4 °C for further studies.

Morphological Characterization

Colony characteristics such as colour, texture, margin, growth rate and pigmentation were recorded. Microscopic observations of hyphae, pycnidia, conidia, asci and ascospores were made using lactophenol cotton blue stain under a compound microscope.

Results

Symptoms

Gummy stem blight was observed to affect watermelon plants at all growth stages, from seedlings to fruit-bearing vines, with symptom severity strongly influenced by environmental conditions such as high humidity and moderate to warm temperatures. In the surveyed fields of Anantharajupeta, Andhra Pradesh, the disease was widespread and caused considerable damage to crop stand and productivity. On leaves, initial symptoms appeared

predominantly on older foliage as small, water-soaked lesions that were circular to irregular in shape. These lesions gradually expanded and turned brown to dark brown, often surrounded by a conspicuous yellow halo. As the disease progressed, multiple lesions coalesced, leading to extensive leaf blighting and premature defoliation. Under severe infection, numerous black, pinhead-sized pycnidia were clearly visible on necrotic leaf tissues, particularly on the abaxial surface Figure (1). Stem and vine infection represented the most diagnostic and destructive symptom of gummy stem blight. Elongated dark brown to black lesions developed mainly near the crown region or at nodal points. These lesions expanded longitudinally and laterally, forming deep cankers that exuded a sticky, amber-coloured gummy substance. Continued disease progression resulted in cracking and girdling of vines, ultimately leading to vine collapse and plant death. In several fields, vine collapse occurred suddenly during the fruiting stage, resulting in significant yield loss Figure (2 and 3).

Fruit infection was commonly observed at the blossom end or at points where fruits were in contact with infected vines or soil. Initial symptoms appeared as small, sunken, brown to black spots, which gradually enlarged and became water-soaked. The affected fruits later developed soft rot accompanied by the appearance of black pycnidia on the surface, rendering them unmarketable. In seedlings, the disease caused damping-off-like symptoms, characterized by necrosis at the collar region, followed by wilting and death.

Isolation and Cultural Characteristics of the Pathogen

The causal organism was consistently isolated from infected leaves, stems, vines, and fruits showing typical gummy stem blight symptoms. Fungal growth was visible on PDA within 2-3 days of incubation at 25 ± 2 °C. Initially, the colonies appeared white to pale grey, with sparse aerial mycelium. As incubation progressed, the colonies gradually darkened to grey and later to dark grey or black due to the formation of pycnidia. Pure cultures obtained through the hyphal tip method exhibited uniform colony morphology, indicating successful isolation of a single pathogen. The fungal colonies showed rapid radial growth, attaining a diameter of 65-80 mm within seven days of incubation. The mycelium was cottony to fluffy in texture, with abundant aerial growth, and the colony margins were irregular to lobate. The reverse side of the culture plates displayed dark brown to black pigmentation, which intensified with age.



Fig 1

Fig 2

Fig 3

Fig 1-3: GSB symptoms on leaf and stem

Pathogenicity Test

Pathogenicity of the isolated fungus was confirmed through artificial inoculation of healthy watermelon seedlings using a conidial suspension adjusted to 1×10^6 spores per millilitre by haemocytometer prepared from actively growing cultures. Inoculated plants developed typical gummy stem blight symptoms within 7-10 days after inoculation. Initial symptoms appeared as water-soaked lesions on leaves, followed by stem lesions with gummy exudation, closely resembling those observed under natural field conditions.

Control plants inoculated with sterile distilled water remained healthy and symptomless throughout the experimental period. The pathogen was successfully re-isolated from the artificially infected plants and exhibited identical cultural and morphological characteristics to the original isolate. Thus, Koch's postulates were fulfilled, confirming *Stagonosporopsis cucurbitacearum* as the causal agent of gummy stem blight of watermelon.

Microscopic and Morphological Characteristics

Microscopic examination revealed that the fungal hyphae were septate, profusely branched, and hyaline to light brown in colour. The hyphal width ranged from 3-6 μm . Pycnidia were abundantly produced in older cultures and appeared as black, globose to sub-globose, thick-walled structures embedded within the mycelial mat. Pycnidia measured 100-300 μm in diameter and possessed a distinct ostiole.

Conidia were hyaline, aseptate, smooth-walled, and ellipsoidal to allantoid in shape, measuring $5-8 \times 1.5-3 \mu\text{m}$. The sexual stage was occasionally observed in aged cultures, characterized by the formation of perithecia containing bitunicate asci. Each ascus contained eight ascospores, which were one-septate, hyaline to light brown and measured $11-16 \times 3-5 \mu\text{m}$ Figure (4). These cultural and microscopic features were consistent with the diagnostic characteristics of *Stagonosporopsis cucurbitacearum*.

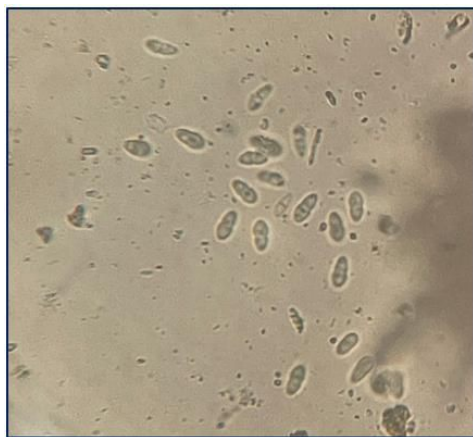


Fig 4: Microscopic observation of Ascospores

Discussion

The present investigation confirmed *Stagonosporopsis cucurbitacearum* as the causal organism of gummy stem blight of watermelon in Andhra Pradesh through detailed symptomatology, cultural and morphological characterization and pathogenicity testing. The disease symptoms observed in the field, including leaf blight, stem cankers with gummy exudation, vine collapse and fruit rot, were in close agreement with earlier reports on gummy stem blight in watermelon and other cucurbits Koike, 1997^[11], Basim *et al.* (2016)^[3], Mahapatra *et al.* (2020)^[13]. The

predominance of stem and vine infection observed in the present study highlights the destructive nature of the disease, as vine cankers directly interfere with nutrient and water transport, leading to sudden plant collapse. The appearance of amber-coloured gummy exudation from infected stems is a hallmark symptom of gummy stem blight and serves as a reliable field diagnostic feature. Pathogenicity tests confirmed the virulence of the isolated pathogen, as artificial inoculation resulted in typical gummy stem blight symptoms within a short incubation period. The successful re-isolation of the pathogen and fulfillment of Koch's postulates conclusively established *Stagonosporopsis cucurbitacearum* as the causal agent. These findings corroborate earlier studies emphasizing the importance of pathogenicity testing in disease diagnosis with the earlier reports of Thomas *et al.* (2012)^[22], Zhao *et al.* (2018)^[23], Savitha and Garampalli (2022)^[19].

The pathogen exhibited rapid growth on potato dextrose agar, producing white to grey colonies that darkened with age due to pycnidial formation. Similar colony characteristics have been reported by Li *et al.* (2015)^[12] and Mahapatra *et al.* (2020)^[13], who described fast-growing colonies with dark pigmentation in *Stagonosporopsis cucurbitacearum*. The production of abundant pycnidia under laboratory conditions further supports the aggressive nature of the pathogen and its ability to survive and spread through asexual spores. Microscopic observations revealed septate hyphae, black ostiolate pycnidia and hyaline, aseptate conidia, which are diagnostic features of *Stagonosporopsis cucurbitacearum*. Occasional observation of the sexual stage, characterized by bitunicate asci and one-septate ascospores, suggests the potential role of sexual reproduction in generating genetic variability and enhancing pathogen adaptability. Similar observations were reported by Li *et al.* (2015)^[12], Huang and Lai (2019)^[8], Basim *et al.* (2016)^[3], Ramsoonder *et al.* (2018)^[17], Das *et al.* (2020)^[7] who have studied the morphological characters of the pathogen.

Conclusion

The present study demonstrates that, isolation and characterization of gummy stem blight of watermelon based on cultural, morphological, and pathogenic characteristics. Infected watermelon plant tissues exhibiting typical gummy stem blight symptoms were collected from affected fields. The pathogen was isolated from diseased tissues and cultured on potato dextrose agar (PDA). Observations on colony morphology, including colour, texture, margin, growth rate and pigmentation were recorded at different incubation periods. The occurrence of severe gummy stem blight in Annamaya district underscores the need for integrated disease management strategies, including the use of resistant cultivars, crop rotation, sanitation, and judicious fungicide application. The identification and characterization of the pathogen form the basis for developing effective disease management and resistance breeding programs.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that no competing interests exist.

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