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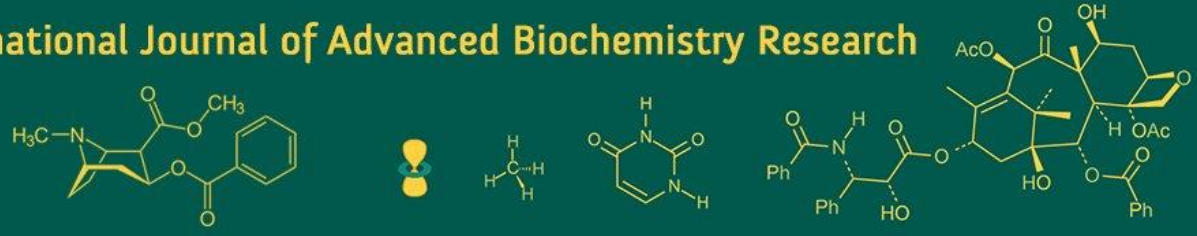

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Isolation, pathogenicity and cultural variability of *Stagonosporopsis cucurbitacearum*

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Abstract
Gummy stem blight is one of the most destructive diseases of cucurbitaceous crops, causing severe yield and quality losses under warm and humid conditions. The disease is primarily incited by *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), a necrotrophic fungal pathogen capable of infecting leaves, stems, and fruits. The present investigation was undertaken to isolate, establish pathogenicity and study the cultural behaviour of *S. cucurbitacearum* on different solid media under *in vitro* conditions. Comparative studies were conducted on five different media *viz*., potato dextrose agar (PDA), corn meal dextrose agar (CMDA), oat meal agar (OMA), V-8 juice agar (V-8A), and host leaf dextrose agar (HLDA) to determine their effect on mycelial growth, colony characteristics, and fructification. Colony morphology varied across media, exhibiting differences in colour and growth pattern. The study highlights PDA and HLDA as the most suitable media for culturing *S. cucurbitacearum*.

Keywords: Gummy stem blight, *Stagonosporopsis cucurbitacearum*, cultural variability

Introduction
Cucurbitaceous vegetables are economically important crops, but their productivity is severely constrained by gummy stem blight (GSB), one of the most destructive fungal diseases causing leaf spots, stem cankers with gummy exudation, vine wilting, and fruit rot under warm and humid conditions. The disease is caused by *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), a seed-, soil-, and residue-borne pathogen. Isolation and pathogenicity testing are essential for confirming disease etiology and fulfilling Koch's postulates. In addition, *in vitro* evaluation of different culture media provides valuable information on mycelial growth, colony morphology and fructification which are crucial for accurate identification and maintenance of the pathogen. Therefore, the present study was undertaken to isolate and confirm the pathogenicity of *S. cucurbitacearum* and to assess the effect of different nutrient media on its growth and fructification under laboratory conditions.

Material and Methods
The pathogen was isolated from infected leaf and stem tissues of ridge gourd using the tissue segment method. Surface-sterilized tissue bits (2-5 mm) from the diseased-healthy junction were plated on potato dextrose agar amended with streptocyclin (30 mg L^{-1}) and incubated at 27±1 °C. The fungus was purified by the single hyphal tip method and maintained on PDA slants at 5 °C for further studies. Pathogenicity was confirmed by spray inoculation of ridge gourd seedlings (3-4 leaf stage) with a spore suspension (1×10^6 spores mL^{-1}). Typical symptoms developed under high humidity, and the pathogen was successfully re-isolated, fulfilling Koch's postulates. For cultural studies, five different solid media *viz*., potato dextrose agar, corn meal agar, V_8 juice agar, oat meal agar and host leaf extract agar were evaluated with respect to their support for growth of the pathogen. Culture discs of 5 mm diameter were lifted with a sterilized cork borer from a 10 days old culture and were aseptically transferred to a petri plate (90 mm diameter) containing test medium and incubated at 25±1 °C. Colony growth and fructification were recorded after 7 and 15 days of incubation respectively.

Category	Fructification	Rating
_	0	Absent
+	1-2	Scanty
++	3-5	Moderate
+++	6-8	Good
++++	>10	Abundant

Results and Discussion

The pathogen was confirmed as *S. cucurbitacearum*. Pathogenicity of the isolate was confirmed by fulfilling Koch's postulates. Typical gummy stem blight symptoms appeared 5-7 days after inoculation, beginning as light brown leaf spots that later coalesced, while control plants remained symptomless. The pathogen was successfully re-isolated on PDA and found to be identical to the original culture, confirming its pathogenic nature.

Comparative studies were conducted on five different media *viz*., potato dextrose agar, corn meal dextrose agar (CMDA), oat meal agar (OMA), V-8 juice agar (V-8A), host leaf dextrose agar (HLDA) to determine the ideal media for attaining the optimum growth and fructification of *Didymella* sp. Average colony diameter and fructification (per 5 mm culture disc) in each medium were recorded after 7 and 15 days of incubation respectively at 25±1 °C. The results revealed that on potato dextrose agar (PDA) and host leaf dextrose agar (HLDA) the growth of the fungal mycelium was maximum (90 mm) after 7 days of incubation. This was followed by V-8 agar (81 mm) and oat meal agar media (67 mm). Minimum mycelial growth was observed on corn meal agar (62 mm).

On potato dextrose agar media, pigmentation varied from white to greyish white and cottony aerial growth was observed. On host leaf dextrose agar media, white to grayish white coloured fluffy mycelial growth was observed. On V-8 agar media, growth of the fungus was fluffy and white in colour. Colour of the fungus on oat meal agar media was white and the growth pattern was fluffy. On corn meal agar media, white and semitransparent growth was observed.

Effect of different media on growth and fructification of *Stagonosporopsis cucurbitacearum*

Agar medium	Colony growth (mm)	Colony colour	Growth pattern	Fructifi cation
Potato dextrose agar	90	White-grayish White	Cottony	+++
Corn meal agar media	62	White	Semi-Transparent	+
V8 juice agar	81	White	Fluffy	++
Oat meal agar	67	White	Fluffy	+
Host leaf extract agar media	90	White-grayish white	Fluffy	+++

The present study confirmed *Stagonosporopsis cucurbitacearum* as the causal agent of gummy stem blight of ridge gourd, based on symptom expression, pathogenicity tests and successful re-isolation of the pathogen, thereby fulfilling Koch's postulates. The appearance of typical gummy stem blight symptoms within 5-7 days after inoculation and the absence of symptoms in control plants clearly established the pathogenic nature of the isolate. The study demonstrated that culture media exert a significant influence on the growth, morphology and fructification of *Stagonosporopsis cucurbitacearum*. The findings are in agreement with earlier reports which indicated PDA as the most suitable medium for culturing the gummy stem blight pathogen. The superior performance of HLDA suggests that host-specific nutrients play an important role in enhancing fungal growth and sporulation. Variations in colony characteristics observed across different media highlight the adaptive nature of the pathogen and emphasize the importance of selecting appropriate media for laboratory studies.

Effect of different media on growth and fructification of *Stagonosporopsis cucurbitacearum*

Agar medium	Colony growth (mm)	Colony colour	Growth pattern	Fructifi cation
Potato dextrose agar	90	White-grayish White	Cottony	+++
Corn meal agar media	62	White	Semi-Transparent	+
V8 juice agar	81	White	Fluffy	++
Oat meal agar	67	White	Fluffy	+
Host leaf extract agar media	90	White-grayish White	Fluffy	+++

Observations recorded after 8 days of incubation at 25±1 °C for growth Observations recorded after 15 days of incubation 25±1 °C for fructification

- = Absent (absent)
+ = Scanty (1-2)
++ = Moderate (3-5)
+++ = Good (6-8)
++++ = Abundant (>10)

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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