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RESEARCH ARTICLE

Gummy stem blight of bottle gourd: Pathogen identification, host screening, and management strategies

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Abstract

Gummy stem blight, caused by *Stagonosporopsis cucurbitacearum* (syn. *Phoma cucurbitacearum*; teleomorph - *Didymella bryoniae*), is becoming a major problem for the cultivation and seed production of bottle gourd, including almost all gourds and melons. During May-June, 2021-2024, severe disease incidence of gummy stem blight occurred on bottle gourd (*Lagenaria siceraria;* Family: Cucurbitaceae) at Indian Council of Agricultural Research – Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh. Its infection is noted on green and ripe fruits and has become considered a serious threat for bottle gourd cultivation and its seed production. Initially, typical symptoms of gummy stem blight appeared as circular dark brown-tan spots at the margin of the leaves and water-soaked areas on the stem near the soil line, including defoliation, wilting, and death of plant vines. The pathogen was isolated from diseased stems and leaves of bottle gourd (var. Kashi Ganga). The pathogen produced white aerial mycelial growth with an undulated colony with cylindrical non-septate to monoseptate conidia. 14-day-old plants of bottle gourd were inoculated by spraying a conidial suspension (2.3×10^6 cfu/mL) of pathogen isolate to test pathogenicity. Based on the detailed cultural, morphological, pathological, microscopic characteristics and pathogenicity test, the causal pathogen was identified as *S. cucurbitacearum*. Twelve cultigens of cucurbits were screened against gummy stem blight pathogen under net-house conditions. On the basis of disease reaction among tested cultigens namely summer fit, cucumber- *Cucumis hardwickii* and bitter gourd (IC-21250044) were found highly resistant. The treatments comprises spot application of *Trichoderma asperellum* + *Bacillus subtillis* (CRB-7) @ 10 gram per pit at the time of planting and soil drenching @1% in the root zone near collar region at the time of each earthing recorded highest *in vivo* seed germination (84%), lowest gummy stem blight percent disease severity index (7.0) and maximum yields (31.66 tonnes/ha) with highest BC ratio (1: 2.73) in comparison to control.

Keywords: Bottle gourd, Gummy stem blight, Disease management, Pathogenicity, *Stagonosporopsis cucurbitacearum.*

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Introduction

Bottle gourd (*Lagenaria siceraria; Family:* Cucurbitaceae) is an economically important vegetable grown in tropical and temperate region of the world. Cucurbits ranked as the second most important family of vegetable crops grown in India. The cultivation of cucurbits gradually expanded and reached up to 0.6 million hectares, including the cultivation of bottle gourd in 0.28 million hectares (Tripathi et al. 2024). From May-June 2021 to 2024, severe disease incidence of gummy stem blight occurred on bottle gourd at the research farm of ICAR-IIVR, Varanasi, Uttar Pradesh, India. Disease incidence afflicted by *Stagonosporopsis cucurbitacearum* was recorded at more than 50% on bottle gourd, ash gourd and cucumber, which poses a major emerging threat to the cultivation of these crops in particular and other cucurbits in general in Northern India (Tripathi *et al.*, 2021; Garmapalli *et al.*, 2016; Sudisha *et al.,* 2004; Al-Jubouri and Hussian, 2020; Sitterly and Keinath, 1996; Robinson and Decker, 1997; Keinath, 2011; CABI Compendium, 2022). Drops of gummy

brown exudates with amber-colored plant sap appear on stems under certain conditions and have the name gummy stem blight. Black dot-like pycnidia are also observed on the infected stems, leaves, and fruits; thus, this black rot stage of the disease is also called black rot. Gummy stem blight (GSB) is caused by *S. cucurbitacearum (*syn. *Phoma cucurbitacearum;* teleomorph - *Didymella bryoniae*) and is an airborne, seed-borne, and soil-borne facultative necrotrophic plant pathogen (Keinath 2011; Lee 1982; Lee et al. 1984). The present study represents the detection, identification, and screening of resistance germplasm of cucurbits for grafting and integrated disease management of an emerging threat of gummy stem blight in bottle gourd.

Materials and Methods

Isolation, detection, identification and inoculum production

Initially, typical symptoms of gummy stem blight appeared as circular dark brown-tan spots at the margin of the leaves and water-soaked areas on the stem near the soil line, including defoliation, wilting, and death of plant vines (Fig. 1). The pathogen was isolated from diseased stems, fruits and leaves of bottle gourd (cv. Kashi Ganga), ash gourd (cv. Kashi Dhawal) and cucumber. Tissues were sterilized with NaOCl @ 1% for one minute and plated onto potato dextrose agar (PDA) Plates. Inoculum prepared on sterilized potato dextrose broth (PDB). A 100 ml sterilized PDB containing flasks were inoculated with 5 ml suspension or 5 PDA plugs (5mm) of actively growing one-week-old culture of pathogen isolate (GSB-1) established from bottle gourd, and the inoculated flasks were incubated at room temperature under 10 hr of fluorescent light per day for 7 days.

Mass screening of resistant genotypes of cucurbits

Twelve genotypes *viz*., bottle gourd (Kashi Ganga, Legacy, VBRTG-61, and Maxima), bitter gourd (IC-2125004), ash gourd (Kashi Dhawal), Cucumber (Summer fit, and Cucumber-*Cucumis hardwickii*), Sponge gourd (Kashi Shreya, VRRG-68-2011, and VRRG-75-2016), and ridge gourd (Kashi Sivani) were screened under pot and through leaf detached methods (St. Amand and Wehner 1995. The spore suspension was standardized to a concentration of 2.3×10^6 conidia per mL by a hemocytometer. The conidial suspension was standardized to a concentration of 2.3×10^6 conidia per mL by hemocytometer (Van Steekelenburg 1981). Pathogenicity was tested on 14-day-old plants of bottle gourd (cv Kashi Ganga) by spraying a pathogen suspension (2.3×10^6 conidia/mL) of a 10-day-old culture of isolate. Before inoculation, WETCIT® (surfactant) was added @ 1.0 ml/L to the conidial

(a) Leaf

(b) Stem

(c) Vine

(d) Fruit

Fig. 1: Typical Symptom of gummy stem blight on bottle gourd

suspension. Control plants were inoculated with sterile distilled water. Inoculated pots were covered by polythene to maintain humidity (> 90%) for 4 days under a green garden shed net. The pathogen was re-isolated from inoculated plants to fulfill Koch's postulate. No disease symptoms were observed on the control plants. Visual ratings of disease development and disease severity on leaves and stems were assessed after 28 days of inoculation. Scoring of disease was done using an index of necrotic tissue by the following visual rating scale (0: no infection visible; 1: > 0% and ≤ 20% leaf area necrotic; 2: > 20% and ≤ 40% leaf area necrotic; 3: > 40% and ≤ 60% leaf area necrotic; 4: > 60% and ≤ 80% leaf area necrotic and 5: > 80% leaf area necrotic). Percent Disease Index (PDI) was calculated based on scoring data of PDI tested germplasm were categorized as: 0 = Immune; 1-10% = Resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75% = susceptible; and >75% = highly susceptible (Gusmini et al. 2005, Dhananjay et al. 2023)

Disease management modules

Seven treatments for integrated disease management of gummy stem blight were formulated *viz.*, T1 = comprises seed treatment by zineb 75% WP @2.5 g/kg seed + soil drenching of zineb @ 0.25% in root zone near collar at the time of first earthing + spray twice of zineb @0.2% at 10 days interval started at first sign of disease; T2= seed treatment by carbendazim 50% @2.5 g/kg seed + soil drenching @0.1% in root zone near collar region at the time of first earthing + spray twice of carbendazim @0.1% at 10 days interval started at first sign of disease; T3= seed treatment by carbendazim 50% @2.5 g/kg seed + application of micronutrients @5 kg/ha in root zone near collar region at the time earthing + foliar spray of azoxystrobin 4.8% + chlorothalonil 40% SC @0.25% at 10 days interval twice at first sign of disease; T4= seed treatment by talc-based consortia of TCV-2 + *Bacillus subtillis* (CRB-7) @10g/kg seed + soil drenching @1% in root zone at the time of each earthing; T5= spot application of *Trichoderma asperellum* + *Bacillus subtillis* (CRB-7) @10 g per pit at the time of planting + soil drenching @1% in root zone near collar region at the time of each earthing; T6 = first spray of carbendazim @0.1% started at first sign of disease followed by spray of zineb @ 0.2% after 10 days of first spray, third spray azoxystrobin 4.8% + chlorothalonil 40% SC @ 0.25% and T7 = control in Randomized Block Design (RBD).

Statistical analysis

The data from field experiments was analyzed using OP Stat (HAU, Haryana). The data was analyzed by simple randomized block design (RBD) and one factor ANOVA in Microsoft Excel. Percentage data was subjected to either angular (if data were mixed values in the range of both < 30 and >70) or square root (if data were either < 30 or >70%) transformations. The significance of the difference between the treatments was tested at 5% probability.

Results and Discussion

Isolation, detection and identification of the pathogen

Gummy stem blight (GSB) pathogen *Stagonosporopsis cucurbitacearum* isolates were established from bottle gourd (var. Kashi Ganga), ash gourd (var. Kashi Dhawal) and cucumber (hybrid Kashi Nutan) for artificial screening of GSB resistant cucurbits. Pure culture of the pathogen isolates produced white aerial mycelial growth with undulated colonies on PDA plates. Inoculated PDA plates produced white aerial mycelial growth with undulated colonies after 7th days of incubation at 24^{o}C with a 12 hours photoperiod (Fig. 2). Mounts were prepared for detection of the pathogen and observed under a microscope. Conidia were cylindrical, non-septate to monoseptate and the average length and width (n = 50) were recorded 60 x 40 µm on Potato Dextrose Agar (PDA) (Keinath, 2011; Lee, 1982; Lee et al. 1984). Conidia were cylindrical, non-septate to monoseptate and the average length and width (n = 50) were 60 x 40 µm on PDA (Fig. 3). Based on the morphological, pathological and microscopic characteristics, the pathogen was identified as *S. cucurbitacearum* (Keinath *et al.* 1995, Sudisha et al. 2004; Choi et al. 2010, Mahapatra et al. 2020).

Gummy stem blight is threatening the cultivation of almost all cucurbit vegetables and their seed crops. In preceding years average gummy stem blight disease incidence was not well documented for this region. However, disease incidence increased from 13 to 21% during 1999 - 2003 in Karnataka (Garampalli 2016). However, its incidence was first time recorded in 1972, 100% in watermelons in 1986, 13% in 1999, 21% in 2003, 41% in seed crop of musk melon in Southern states of India, covering a total area at the national level 1.03 million hectare (Garmapalli et al. 2016). Earlier farmers cultivated cucurbits for self-consumption but now adopting it as a cash crop for commercial cultivation as well as seed production. Recently, growers used bottle gourd as disease-resistant rootstock for the grafting of cucumber and bitter gourd for the management of soil-borne disease. Disease incidence afflicted by *S. cucurbitacearum* was recorded more than 50% on almost all the cucurbits such as ash gourd, bottle gourd, bitter gourd, cucumber, pumpkin, ridge gourd, muskmelon and watermelon, which poses major emerging threat for cultivation of cucurbits in Northern India (Garmapalli et al. 2016, Tripathi et al. 2021).

Screening of resistant genotypes

Twelve genotypes of cucurbits were screened against the gummy stem blight pathogen in a pot under a green garden shed net. Visual ratings of disease development and disease severity index were assessed on leaves and stems of the plants after 28 days of inoculation (Fig. 4) (Sudisha *et al.* 2004, Keinath 2011, Garmapalli *et al.* 2016, Tripathi *et al.* 2021).

Fig. 2: PDA culture plate of *S. cucubitacearum*

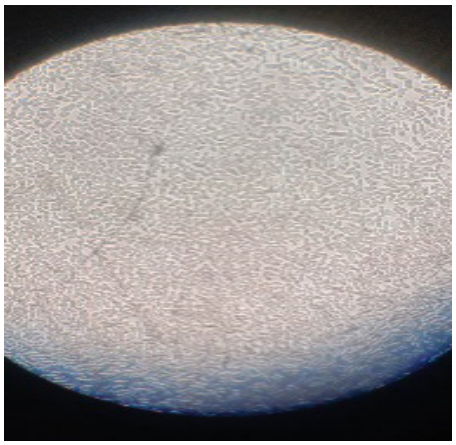

Fig. 3: Microphotograph of conidia of *S. cucubitacearum*

Based on the disease reaction, genotypes were categorized in two groups i.e., resistant *viz.*, cucumber (Summer fit, and cucumber- *C. hardwickii*) and bitter gourd (IC-2125004), and susceptible *viz.*, bottle gourd (Kashi Ganga, Legacy, VBRTG-61, and Maxima), ash gourd (Kashi Dhawal), Sponge gourd (Kashi Shreya, VRRG-68-2011, and VRRG-75-2016), and ridge gourd (Kashi Sivani).

Fig. 4: Pathogenicity test - Inoculated and Control plant

Integrated disease management

Gummy stem blight disease first time appeared in the field on leaves and stems after 90 days of sowing. Among the various treatments for the management of this pathogen, T5 responded the best in terms of the highest *in vivo* seed germination (84%), the lowest gummy stem blight percent disease severity index (7.0), and maximum yield (31.66 ton/ha) with highest BC ratio (2.73). The treatment (T5) comprises spot application of *Trichoderma asperellum* + *Bacillus subtillis* (CRB-7) @10 g per pit at the time of planting + soil drenching @1% in root zone near collar region at the time of each earthing) in comparison to control (Table 1).

Gummy stem blight is an emerging fungal disease of cucurbits worldwide (Garmapalli *et al.* 2016). Its incidence might have occurred due to changes in production practices, increasing acreage under cucurbits hybrids and the appearance of highly variable climate change. Due to unexpected rains during the summer season and, temperature of 42°C and high relative humidity of 85%, including the vulnerability of the crop varieties, monocropping, failure of host resistance and failure of applied fungicide efficacy, the appearance of fungicide resistance in pathogen and emergence of new virulent/

Table 1: Effect of various treatments on gummy stem blight in bottle gourd

Treatments	*Germination (%)*			*Mean*	*Disease severity (PDI)*			*Mean*	*Yield (tonnes/ha)*			*Mean*	*BC Ratio*
	2022	*2023*	*2024*		*2022*	*2023*	*2024*		*2022*	*2023*	*2024*		
T-1	86.00	68.51	79.49	78.00	25.00	11.00	9.99	15.33	20.00	16.32	20.68	19.00	1.57
T-2	78.00	62.95	63.05	70.00	16.00	3.10	11.89	10.33	36.25	30.39	19.34	28.66	2.44
T-3	80.00	64.81	71.19	72.00	16.00	4.00	4.00	8.00	36.25	23.60	32.13	30.66	2.63
T-4	80.00	70.36	74.64	75.00	16.00	10.10	14.88	13.66	23.00	24.73	24.27	24.00	2.05
T-5	90.00	77.77	84.23	84.00	5.00	9.99	6.01	7.00	32.00	29.46	35.52	31.66	2.73
T-6	85.00	74.06	77.94	79.00	16.00	5.55	6.44	9.33	23.00	27.86	25.13	25.33	2.15
T-7	72.00	59.25	64.74	65.33	33.00	12.21	11.79	19.00	19.00	14.53	16.45	16.66	1.28
CD (5%)				3.53				3.16				3.02	-
SE (m)				1.13				1.01				0.97	-
CV (%)				2.62				14.89				6.68	-

aggressive strain of pathogen might be involved for severity of diseases under changing production system/ cropping scenario. This production volume changes due to the government policies for crop diversification, promotion of vegetable export and commercial production for vegetable and seed requirements. An increasing trend in production (24.34%) and cultivation area expansion (16.41%) were observed. Cucurbit production volume changes due to government policies for crop diversification, promotion of vegetable export, and commercial production for vegetable and seed requirements. Disease incidence might have occurred due to changes in production practices, increasing acreage under cucurbits, and the appearance of highly variable climate change, including vulnerability of the crop varieties, monocropping, failure of host resistance, failure of applied fungicide efficacy, appearance of fungicide resistance in pathogens, and emergence of new virulent/ aggressive strains of pathogens, which might be involved in the severity of diseases under changing farming systems and production situations (Sitterly and Keinath 1996, Robinson and Decker 1997). Thus, the report highlights the emergence of this pathogen in Uttar Pradesh, India, which will contribute to the screening of disease resistance cultigens of cucurbits and breeding for disease resistance varieties/rootstock for disease management.

Conclusion

The present study records an emerging pathogen threat of gummy stem blight (*S. cucurbitacearum*) on bottle gourd in Eastern Uttar Pradesh, India. It is noted as a soil- and seed-borne disease, but its average incidence on cucurbits has not been well documented for this region. Thus, the report highlights the emergence of this pathogen in India, which will contribute to the screening of disease resistance cultigens of cucurbits and breeding for disease resistance varieties/rootstock for its management.

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सारांश

चिपचिपा तना झुलसा रोग स्टैगोनोस्पोरोप्सिस कुकुरबिटेसीरम (फोमा कुकुरबिटेसीरम; टेलोमॉर्फ - डिडिमेला ब्रायोनिया) के कारण होता है, जो लगभग सभी लौकी और खरबूजे की खेती और बीज उत्पादन के लिए एक समस्या है। मई-जून, 2021-2024 के दौरान, भारतीय कृषि अनुसंधान परिषद - भारतीय सब्जी अनुसंधान संस्थान, प्रायोगिक फार्म और वाराणसी, उत्तर प्रदेश के आसपास के किसानों के खेतों में लौकी (लेगेनेरिया सिसेरिया, कुकुरबिटेसी) पर चिपचिपे तना झुलसा रोग का गंभीर प्रकोप हुआ । प्रारंभ में, चिपचिपे तना झुलसा रोग के विशिष्ट लक्षण पत्तियों के किनारे पर गोलाकार गहरे भूरे रंग के धब्बे और मिट्टी की रेखा के पास तने पर दिखाई देते है। जिसमें पत्ते झड़ना, मुरझाना और पौधे की लताओं का मरना शामिल था। रोगज़नक़ को लौकी (किस्म काशी गंगा) के रोगग्रस्त तनों और पत्तियों से अलग किया गया। रोगज़नक़ ने बेलनाकार गैर-सेप्टेट से मोनोसेप्टेट कोनिडिया और लहरदार कॉलोनी के साथ सफ़ेद हवाई माइसेलियल वृद्धि का उत्पादन किया। रोगज़नक़ का परीक्षण करने के लिए रोगज़नक़ के कोनिडियल सस्पेंशन का छिड़काव लौकी (किस्म काशी गंगा) में किया गया। विस्तृत रोगात्मक विशेषताओं के आधार पर रोगज़नक़ की पहचान एस. कुकुरबिटेसीरम के रूप में की गई। गमी स्टेम ब्लाइट रोगज़नक़ के प्रति कुकुरबिट की किस्मों की जाँच की गई। परीक्षण की गई किस्मों में रोग प्रतिक्रिया के आधार पर समर फिट, हार्डविकी और करेला IC-21250044 अत्यधिक प्रतिरोधी पाए गए। उपचार में रोपण के समय ट्राइकोडर्मा एस्परेलम + बैसिलस सबटिलिस (सीआरबी-7) @ 10 ग्राम प्रति गड्ढे की दर से छिड़काव और प्रत्येक मिट्टी चढ़ाने के समय कॉलर क्षेत्र के पास जड़ क्षेत्र में 1% की दर से मिट्टी को भिगोना नियंत्रण की तुलना में उच्चतम इन विवो बीज अंकुरण (84%), सबसे कम गमी स्टेम ब्लाइट प्रतिशत रोग गंभीरता सूचकांक (7.0) और उच्चतम बीसी अनुपात (1: 2.73) के साथ अधिकतम उपज (31.66 टन/हेक्टेयर) दर्ज की गई।