Incidence of *Alternaria* fruit rot as a latent postharvest disease in guava (*Psidium guajava* L.) in Kerala.

ABSTRACT

A purposive random sampling survey was conducted to study the incidence of latent pathogens associated with postharvest losses in guava sourced from markets and homesteads of Ernakulam, Thrissur and Palakkad districts of Kerala during 2024. Fruits at mature green stage were collected then surface sterilized and incubated at room temperature for symptom development. After three to five days of incubation, few of the fruits collected from Thrissur district started development of dry brown lesions. The disease developed under high humidity and temperature between 30 and 35 °C. Four isolates of *Alternaria* sp. were isolated from the fruits. The highest disease incidence recorded was 75 % from Vettikkal while the highest severity of 20 % was recorded in fruits collected from Shakthan Market. The isolated pathogen appeared white to grey coloured, submerged to aerial mycelia which showed uniform and zonal growth at regular intervals. The isolates exhibited an average growth rate of 0.6-0.9 cm per day. The hyphae were brown-coloured, septate with straight or curved, geniculate, pale brown conidiophores bearing a branched chain of conidia. The conidia were brown, obclavate in shape with 3-4 transverse and 1-2 longitudinal septa having average size of 22.08 to 29.53 × 7.85 to 12.54 µm. Molecular characterization using ITS primers followed by sequence analysis in the NCBI database, identified the pathogen as *Alternaria alternata*.

Keywords: guava, postharvest diseases, fruit rot, Alternaria alternata

1. INTRODUCTION

Guava (*Psidium guajava* L.), a member of the Myrtaceae family, is native to South America (specifically Peru) and is widely cultivated in tropical and subtropical regions due to its hardiness and adaptability. It contains high amounts of vitamins A, B1, B2, dietary minerals, lutein, zeaxanthin, lycopene, folic acid, potassium, copper, and manganese (Rahman *et al*., 2003). Guava is also a rich source of vitamin C and ranks second to aonla in terms of vitamin C content. As one of the most important fruit crops in terms of area and production, after mango, banana, and citrus, guava plays a vital role in human nutrition and health. However, despite its nutritional benefits, guava's perishable nature makes it highly susceptible to significant postharvest losses. One of the major contributors to these losses is fungal infection, which can occur at various stages of the fruit's lifecycle, including during the growing season, harvesting, handling, transport, post-harvest storage, and marketing.

Alternaria alternata, is a destructive pathogen which is responsible for various diseases during the postharvest life of many horticultural crops. For instance, *Alternaria* causes stem-end rot in mangoes (Amin *et al*., 2011), fruit spot on apples (Harteveld *et al*., 2013), black rot in kiwi fruit (Kwon *et al*., 2011), and *Alternaria* rot on figs (Doster and

Michaillides, 2007). Studies by Prusky *et al*. (2002) have shown that *Alternaria alternata* can remain quiescent on the cuticles of unripe mango and avocado fruits but later colonize the mesocarp of these fruits during ripening. This suggests that specific changes occurring during fruit ripening may trigger the transition to active infection. Similarly, *Alternaria* fruit rot has been reported in guava, with Srivastav and Lal (2009) from fruit markets of Allahabad. Nongmaithem (2014) and Fatima (2019) also reported *Alternaria* fruit rot in guava. Fungi like *Alternaria* are capable of sporulating and resisting postharvest treatments, enabling them to infect fruits even during transportation to international markets. Given the significant impact of *Alternaria* infections on postharvest quality of guava fruit, this study aims to document the incidence and severity of *Alternaria* fruit rot in guava in Ernakulam, Thrissur and Palakkad districts in the areas delineated under agroecological units AEU 10 (North Central Laterites) and AEU 12 (Southern Foothills) of Kerala, India. Additionally, the study will examine the disease development in guava under laboratory conditions and the cultural-morphological characteristics and pathogenicity of the pathogen.

2. MATERIAL AND METHODS

2.1 SURVEY AND COLLECTION OF FRUIT SAMPLES

The study was conducted during 2024 at the Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala. Purposive random sampling surveys were carried out in homesteads and markets of nineteen different locations in Ernakulam, Thrissur and Palakkad districts delineated under agroecological unit AEU 10 (North Central Laterites) and AEU 12 (Southern Foothills) of Kerala from January to September 2024. The surveys were conducted in eight locations in Ernakulam and Thrissur districts and three locations in Palakkad district. Apparently, healthy fruits (1 kg) were collected from different places, surface sterilized with 1 % sodium hypochlorite solution for 5 min and washed three times with sterile distilled water then incubated at room temperature for disease development. The disease symptoms that developed were extensively examined and documented to understand the expression and progression of latent postharvest diseases under natural conditions. Disease incidence and severity were assessed using the formulae given below and expressed as percent disease incidence and percent disease severity (Lakshmi *et al*., 2011; Wheeler,1969).

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of fruits infected}}{\text{Total number of fruits}} \times 100$$

$$\text{Percent disease severity} = \frac{\text{Sum of disease ratings}}{\text{Total number of samples assessed} \times \text{maximum disease grade}} \times 100$$

Table 1. Disease rating scale (Lakshmi *et al*., 2011)

Grade	Fruit area infected
0	No infection
1	0-5%
2	6-10%
3	11-20%
4	21-50%
5	50%

2.2 ISOLATION OF PATHOGEN AND PATHOGENICITY TESTS

The pathogens associated with symptomatic fruit specimens were isolated using the tissue segmentation method (Rangaswamy, 1958). The fruits were surface sterilized using 1 % sodium hypochlorite solution for 3 min and washed thrice with sterile distilled water. After disinfection, the diseased fruit sample bit was cut into small pieces of 5 mm and placed over PDA media. After incubation, the hyphal tips of the fungal colonies were transferred aseptically to fresh PDA media in Petri plates. The isolates were named according to the geographic origin of the sample using the initial letter of the district name followed by starting letter of the location name and concluding with G1, G2, and so forth for each isolate. The fungal cultures were maintained in PDA slants and stored under refrigeration (4 °C) for future studies. The pathogenicity of fungal isolates was tested to prove the Koch postulates. The mycelial bit inoculation method explained by Rocha *et al*. (1998) was followed for the inoculation of pathogens to confirm pathogenicity. The healthy, semi-ripe guava fruits were surface sterilized by dipping in sodium hypochlorite (1 %) for 15 min followed by three washings with sterile distilled water. Mycelial bits taken from the margins of actively growing colonies (seven days old culture) of the pathogen were placed on healthy fruits. The fruits were pinpricked with a sterile dissection needle before inoculation to facilitate better pathogen establishment. Fruits without pinpricks were also inoculated, and both types were kept under room conditions for disease development. Control was maintained using a PDA disc that was free of pathogens. Moist cotton swab was placed over the mycelial bits or PDA disc. The fruits were then covered in polypropylene cover and maintained at room temperature for symptom development. When the symptoms appeared, the pathogen was re-isolated from the symptomatic fruits into PDA media. The re-isolated pathogen culture was compared with the original culture to prove Koch's postulates.

2.3 CHARACTERISATION OF PATHOGENS

The cultural characteristics of the pathogen in terms of growth, viz., texture, colour, growth rate, sporulation, and reverse side colour on Petri plates were observed. The morphological identification of the fungus was done based on texture, pigmentation, characteristics of conidia in comparison with standard descriptors. Molecular characterization was used to confirm the species of the isolated pathogen. The genomic DNA was extracted using the modified protocol of Doyle (1991), sequences were amplified using ITS primers, ITS-1F and ITS-4R (White *et al*., 1990) followed by sequencing and coupling using in silico cap3 contig assembly and processed through the BLASTn at NCBI database.

3. RESULTS AND DISCUSSION

3.1 DISEASE INCIDENCE AND SYMPTOMATOLOGY

Alternaria fruit rot, a latent postharvest disease of guava, was observed after three to five days of incubation in fruit samples collected from Vettikkal (TVG 1, TVG 2, TVG 3) and Shakthan market (TSG 1) in Thrissur district during March and April 2024 (Table 2). Fruit rot was observed in four out of twenty-four fruit (local and Thailand varieties) samples collected from the Thrissur district, resulting in an incidence of 16.6 %. The sample collection sites were delineated within AEU 10 (North Central Laterites) which is characterized by a tropical humid monsoon climate and a dry period of around four and half months. The fruit samples were collected during the dry period when diurnal temperature fluctuations were recorded between 23.1 and 40 °C and relative humidity varied from 23 to 95 %. The disease developed under incubation at high humid conditions 90-95% and temperature between 30 and 35 °C as light brown to brown irregular lesions, ranging from 0.5 to 3 cm in diameter (Fig.1.). The symptoms on samples TVG 2 and TVG 3 appeared after three days of incubation, and that on TSG 1

and TVG 1 appeared after four and five days of incubation respectively. At the sampling locations, the Percent Disease Incidence (PDI) and Percent Disease Severity (PDS) of *Alternaria* fruit rot recorded were between 33.3-75% and 5-20% respectively. The highest PDI of 75% was observed in fruits collected from Vettikkal, while the highest PDS of 20% was recorded in fruits collected from Shakthan Market. The disease was not recorded from other seventeen locations surveyed in Kerala. In a survey conducted by Srivastava and Lal (2009) in fruit markets of Allahabad, the incidence of 17.69% *Alternaria* fruit rot in guava was recorded. Jha and Chourasia (2021) also recorded 16.8% fruit decay caused by *Alternaria* during storage under ambient conditions with 18-22 °C and 75-80% RH at Bhagalpur during 2014-2016. Maiti and Lal (2024) reported 11.42% average incidence of *Alternaria* fruit rot in Prayagraj during October 2023 to February 2024. In this study, despite the higher temperature of 30-35 °C, disease development was observed under high humidity conditions. But the elevated temperatures might have reduced the rate of symptom progression. Brown irregular lesions initially developed on the fruit epidermis extended into pulp as the symptoms progressed. Nongmaithem (2014) and Maiti and Lal (2024) observed that *Alternaria* fruit rot in guava, initially caused brown dry or parched spots on the fruit epidermis that subsequently developed into circular to oval or irregular grey-brown lesions that invade the inner pulp at later stages. Similar kinds of symptom development were noticed in this study.

TVG 1 **TVG 2** **TVG 3** **TSG 1**

Fig. 1. Symptoms of *Alternaria* fruit rot on guava

Table 2. Samples showing *Alternaria* fruit rot symptoms and days taken for the appearance of symptom, per cent disease incidence, and per cent disease severity

Sl. No.	Samples	Date of survey	Symptoms	Incubation period (DAI)	Percent disease incidence (PDI)	Percent disease severity (PDS)
1.	TVG 1	07-03-2024	Numerous small, light brown, irregular lesions with different diameters ranging from 0.3 to 1.5 cm	5	75	10
2.	TVG 2	07-03-2024	Irregular, brown lesions with different diameter ranging from 0.5 to 1.5 cm on the skin of the fruit	3	75	5
3.	TVG 3	07-03-2024	Brown, irregular, small lesion of 0.5 cm diameter	3	75	5
4.	TSG 1	16-04-2024	Brown discolouration with a diameter of 1.5 cm on one side of the fruit	4	33.3	20

3.2 ISOLATION OF PATHOGEN AND PATHOGENICITY TESTS

The pathogen was isolated from symptomatic areas of fruit rot samples on potato dextrose agar (PDA) medium. On PDA, the pathogen produced white to grey-coloured aerial mycelia. The isolates were named according to the geographic origin of the sample, using the initial letter of the district name followed by initial letter of the location name and concluding with G1, G2, and so forth for each isolate. Thus, the isolates obtained from samples collected from Vettikkal and Shakthan market were named as TVG 1, TVG 2, TVG 3, and TSG 1 respectively. Seven days old pure cultures of the pathogen isolates were used to test pathogenicity. Symptoms started to appear on pinpricked fruits where the mycelial bit inoculum was covered with cotton swab within three to six days of inoculation, but the fruits without pinpricks remained asymptomatic. On these fruits *Alternaria* fruit rot pathogen isolates TVG 1, TVG 2, TVG 3, and TSG 1 resulted in the development of circular to irregular, brown, sunken lesions of 0.6-2 cm diameter on the surface. Notably, the lesion size progression was significantly slower (Fig. 2.). The symptoms were identical to those seen on naturally infected fruits. Previous studies also reported the pathogenicity of *A. alternata* on guava fruits, when inoculated with spore suspensions of 1×10^4 spores/ml (Nongmaithem, 2014) and 1×10^6 spores/ml (Youssef *et al*., 2015). Among the different pathogen isolates, TVG 3 and TSG 1 produced symptoms within four days after inoculation while TVG 2 took five days and TVG 1 took six days to express symptoms on inoculated fruits. However, the isolate TVG 1 exhibited rapid progression of symptoms. *Alternaria* is generally considered as a weak pathogen that enters plants through wounds or natural openings and remains quiescent in fruits until ripening (Pearson and Hall, 1975). Therefore, the slower symptom expression may be because of the green ripe fruits used for inoculation rather than ripe fruits.

TVG 1 – 7 DAI* **TVG 2 – 5 DAI** **TVG 3 – 4 DAI** **TSG 1 – 4 DAI**

Control

*DAI – Days after inoculation

Fig. 2. Symptoms of Alternaria fruit rot of guava upon artificial inoculation at room temperature

3.3 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

The pathogen isolates initially developed white to grey coloured, submerged to aerial fluffy mycelia which showed uniform and zonal growth at regular intervals with grey or greyish black center and prominent white margins. The mycelia completely turned into greyish black later. The reverse side of the plate shows a cream colour which later turns brown (Fig. 3.). The mycelia covered 9 cm plate after 10-13 days of incubation with an average growth rate of 0.6-0.9 cm per day (Table 3). The isolates TVG 2 and TVG 3 showed higher growth rates, i.e., 0.9 cm per day. The hyphae were brown-coloured, septate with straight or curved, geniculate, pale brown conidiophores bearing a branched chain of conidia. The conidia were brown, obclavate in shape with 3-4 transverse and 1-2 longitudinal septa having an average size of 22.08 to 29.53 × 7.85 to 12.54 µm (Fig. 4.). The pathogen isolates TVG 1, TVG 2, TVG 3 and TSG 1 associated with guava fruit rot were identified as *Alternaria* sp. Similar morphological characteristics were documented by Simmons and Roberts (1993) for *Alternaria* sp. causing black spots in Japanese pear.

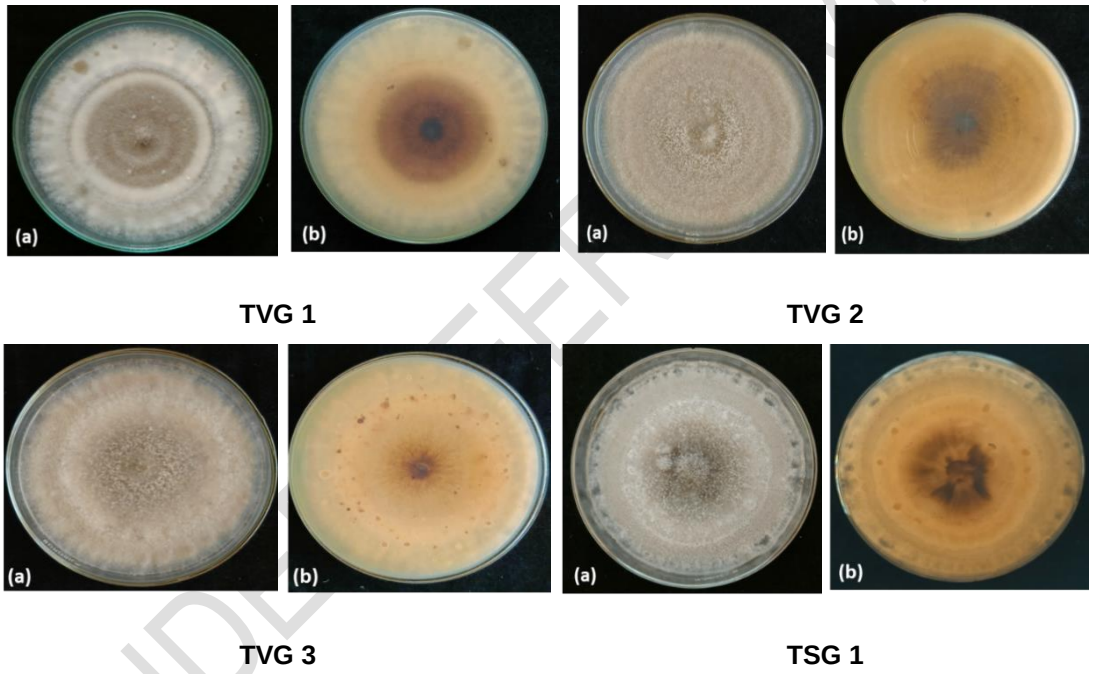

Fig. 3. Colony morphology of pathogen isolates on PDA after 10-15 days (a) Front view of the colony (b) Reverse view of the colony

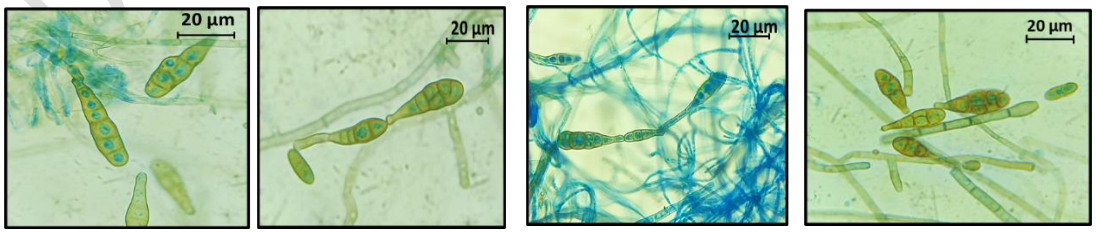

Fig. 4. Conidia of different *Alternaria* isolates (1000 X)

Table 3. Cultural and morphological characteristics of isolates

Sl. No.	Isolate	Cultural characters	Reverse side of the Petri plate	Dimension of conidia (µm)	Average growth rate (cm per day)
1.	TVG 1	White, submerged mycelia with grey at the center shows diurnal zonation, eventually turned to a greyish brown	Off-white colour with brown center	22.08 to 29.53 × 7.65 to 12.54	0.81
2.	TVG 2	Whitish grey, fluffy mycelia with regular and uniform growth, later colony turned brown.	Cream colour	20.25 to 26.54 × 6.87 to 12.50	0.9
3.	TVG 3	Fluffy, dull whitish-grey mycelium which showed uniform and zonal growth at regular intervals	Cream colour which later turns to brown	20.64 to 29.38 × 7.87 to 11.87	0.9
4.	TSG 1	Fluffy, upraised whitish grey mycelium with zonal growth at regular interval	Cream with the brown colour center	22.29 to 26.12 × 7.87 to 11.24	0.6

Alternaria is well known for exhibiting morphological plasticity under different conditions (Simmons, 1992). Therefore, species level identification of the pathogen isolate *Alternaria* sp. (TSG 1) was done by comparing the amplified ITS sequences (ITS 1F and ITS 4R) of the isolate with that in the NCBI database. The result showed 99.1 per cent similarity and 98 per cent query cover with *Alternaria alternata* with accession number MK518438.1 which was isolated from *Basella alba* leaf as an endophyte from Karnataka (Fig. 5). This confirms the identity of the isolate as *Alternaria alternata*. The sequence was deposited in GenBank of NCBI with the accession number PQ763964.

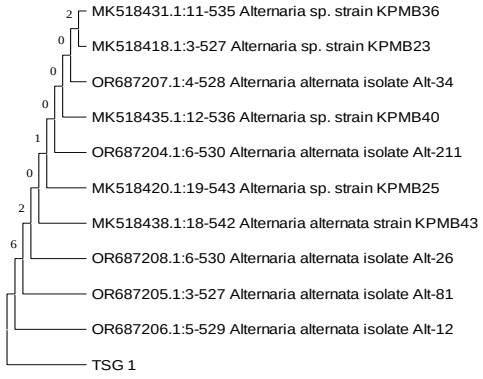

Fig. 5. Maximum likelihood phylogenetic tree produced using multiple alignment of ITS gene sequence of TSG 1 isolate with related species found in NCBI GenBank database

4. CONCLUSION

The prevalence of *Alternaria alternata*, the fungal pathogen responsible for guava fruit rot, was observed in Thrissur district, one of the three districts surveyed in this study. Temperature and relative humidity appear to influence the incidence of *Alternaria* fruit rot. The infection, which may have occurred before or after harvest, remained dormant in the fruits until incubation under high humidity near saturation. This study confirms previous research that highlighted the prevalence of the disease and associated losses at different locations, emphasizing the impact of this pathogen on the post-harvest life of guava. Losses due to postharvest decay remain a major economic concern. Therefore, the results obtained from this study could be useful in planning phytosanitary control strategies from the field to storage to reduce yield loss.

Disclaimer (Artificial intelligence)

Details of the AI usage are given below

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have not been used during the writing or editing of manuscripts.

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