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Prevalence and Pathogenicity of Guava Anthracnose with Special Emphasis on Varietal Reaction

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Abstract: Guava anthracnose was found more prevalent during the main season (April-September) than in off-season (November-February). *Pestalotiopsis psidii*, *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were established as causal organisms of guava anthracnose. On potato dextrose agar medium, the growth of *P. psidii* was observed to be too slow as against the very quick growth of *B. theobromae*. All the pathogens grew well at 28 and 30°C except only the *P. psidii* at 30°C. No varieties were found resistant against all three pathogens tested but local variety I, II and IV proved resistant only against *Pestalotiopsis psidii* both *in vitro* and *in situ*. The Pear shaped fruits had less susceptibility than elliptical round fruits. Local varieties were less susceptible than commercial ones. In inoculated condition, variety kanchannagar showed less susceptibility in comparison with the variety sarupkati and kazipayara. Immature fruits of local variety showed decline in ascorbic acid content when diseased.

Key words: *B. theobromae*, *C. gloeosporioides*, fruit anthracnose, guava, pathogenicity, *P. psidii*, resistance, variety

Introduction

Guava (*Psidium guajava*) the vitamin C enrich fruit plant grown abundantly throughout Bangladesh even with any or little care, mainly in the backyards, except in Kanchannagar of Chittagong district and Sarupkati of Perozpur district where it is cultivated commercially. Among the tree fruit plants guava starts bearing within the shortest possible time and produce abundant fruits. A total of 10 diseases have been reported on guava of which anthracnose is recognized as the second most important disease. High prevalence of the disease has been reported (Meah and Khan, 1987; Rahman and Hossain, 1989; Anonymous, 1990). The disease becomes a serious obstacle to guava cultivation, food values and market price are falling and cause a great threat to germplasm preservation. The farmers think to avoid the cultivation of guava owing to a great loss by this disease. So, work is necessary to protect the nutritious and highly productive guava fruits from anthracnose.

Various approaches including chemical sprays (Rahman and Hossain, 1989; Hossain and Meah, 1992) and cultural practices (Rahman and Hossain, 1989; Ansari, 2000) have been launched to control anthracnose but with partial success. There is no available information on resistant source. However it has been known that certain guava varieties might contain anti-pathogen chemical in the

skin/flesh (Shukla, 1972). Very little or no work has been done on this line. Therefore the present work was undertaken to explore the possibilities of existence of resistance in the indigenous germplasms and biochemical basis of the resistance if any. Moreover isolation, identification and pathogenically of the causal organism were also studied.

Materials and Methods

Prevalence of disease and study on variety: Incidence of guava fruit anthracnose was surveyed in Perozpur, Sylhet and Mymensingh, the three guava producing regions in Bangladesh. Nearly 60 gardens at five locations (Sarupkati, Adabari, Adomkati, Kuriana, Mahmudkati) in Perozpur district were inspected during main season (April-September) in 1990. In Mymensingh, Bangladesh Agricultural University (BAU) Campus, Horticulture Base at Kawatkhali and BADC-farm at Muktagacha were the three spots inspected during both off season (November-February, 1990-91) and main season (1990). While Jaintapur fruit farm and BARI sub-station fruit garden in Sylhet were inspected for guava anthracnose in the same seasons and years. Disease occurrence and severity was observed on guava varieties as it was available under different survey areas within the time from post flowering to mature fruit stage. The local varieties under this study

were categorized on the basis of their characteristics. Commercially important variety kazipeyara, sarupkati and kanchannagar were also studied characteristically.

Isolation and Identification of the causal organisms:

Diseased leaf, twigs and fruits of different maturity stages were collected under survey in different seasons from different areas of Bangladesh. Inocula prepared from diseased specimen were transferred to potato dextrose agar (PDA) plates for isolation of the causal organisms following the tissue planting method (Hossain, 1989). Pure-culture of the organisms were prepared by transferring single spore or mycelium to PDA plates and identified.

Growth of the organisms: Linear growth of the causal organisms of guava fruit anthracnose in potato dextrose agar medium was measured at 24 h interval incubated at four different temperatures were 15, 28, 30 and 35°C.

Evaluation of guava varieties against the disease:

Reaction of different guava varieties to anthracnose causal organisms were evaluated under both natural and laboratory conditions. Tested local varieties were I, II, IV, VII and Commercial varieties were sarupkati, kanchannagar and kazipayara.

In situ inoculation: Fresh immature and mature guava fruits were inoculated when the fruits were intact to the twigs of the standing plants by both pricking and without pricking (Tandon and Singh, 1969). Inoculations were done by fungal block (mycelia, mycelia and spore) over pricked and unpricked spot on the fruit. The inocula were given wet cotton wool covering and covered with moist polyethylene bag. Fruits pricked and unpricked and covered with wet cotton wool but not inoculated by fungal block served as control.

In vitro inoculation: Four immature and mature fresh guava fruits intact to the twigs were placed in the conical flask containing sterile water when the distal end of the twigs immersed in water. Fruits were inoculated with one single organism in the way it was done *in situ* inoculation. All the plant parts were covered with moist polyethylene bag for 48 hours after inoculation. After inoculation observations on the development of infection were made. Inoculations both *in situ* and *in vitro* were done following the procedures of Hossain (1989).

Ascorbic acid: A comparative estimate of ascorbic acid of some selected varieties were done between diseased and healthy fruits (both immature and mature)

following visual titration method based on reduction of 2, 6- dichlorophenol indophenol dye. Ascorbic acid was extracted from infected and healthy guava fruits and estimated by titrimetric method (visual titration) (Reo, 1954). Guava fruits were cut into 2-3 mm pieces. A 5 g of cut pieces were placed in the blender and added metaphosphoric acid at the rate of 4 ml for each guava fruit. The fruit tissues were crushed for 5 min. The extract was filtered through two layers of cheese-cloth. After filtration, the filtrate was centrifuged for 40 min. Then final volume was made with metaphosphoric acid to present 1 g fruit tissue in 5 ml of the solvent. Five ml of the metaphosphoric acid extract was pipetted to a white porcelain dish and titrated against the standardized indophenol reagent (2,6-dichlorophenol indophenol). Amount of ascorbic acid was calculated by the following formula-

$$(1 \times S \times D)/A \times 100/W = \text{mg of ascorbic acid}/100 \text{ g tissue,}$$

Where, 1= ml of indophenol reagent used in titration, S= mg of ascorbic acid reacting with 1 ml of the reagent, D= Volume of the extract (ml), A= Aliquot titrated (ml) and W= Weight of the sample (g).

Results

Natural prevalence of anthracnose: At BAU-Campus, the disease incidence in seven local varieties were almost absent during the off season (OS) of 1990-91, but 40-100% plants were infected during the main season (MS) of 1990 when 10-30% leaves, 30-70% fruits were infected and flowers were found not infected. Local variety VII was observed to carry more infections in both the seasons than others (Fig. 1).

In Perozpur region, an average of 17.4% leaves, 13% twig and 52% fruits of variety sarupkati were diseased by anthracnose. Disease incidence observed in five spots of Perozpur where 2-40% leaves, 0-30% twig and 10-80% fruits were infected during main season in 1990 (Fig. 2).

During the OS of 1990-91, anthracnose was absent in kazipayara and 2% plant infected in sarupkati variety at Horticulture Base. In the MS, more than 50% plants in which 20% fruits of kazipayara and 80% plants of sarupkati variety in which 20% fruits were infected by anthracnose (Fig. 3). Both kazipayara and kanchannagar varieties were found unaffected during OS and a very low (negligible) incidence of anthracnose was observed in the MS in BADC-farm, Muktagacha. At Jaintapur BARI fruit farm, the variety kanchannagar was also found free from anthracnose during OS and incidence of anthracnose was reported to be negligible during the MS of 1990.

Table 1: Fruit characteristics of guava varieties cultivated in Bangladesh

Name of the varieties	External characteristics			Internal characteristics		
	Shape	Size	Color	Seed status	Flesh color	Texture
Local I	Pear shape	Medium	Light green	Medium	White	Crisp
Local II	Pear shape	Medium	Light green	Medium	White	Compact
Local III	Round	Medium	Green	Much	White	Compact
Local IV	Elliptical	Small	Whitish green	Much	Red	Compact
Local V	Elliptical to round	Medium	Green	Much	White	Compact
Local VI	Pear shape	Medium	Green	Few	White	Semi losse
Local VII	Round	Medium	Deep green	Much	Whitish	Compact
Sarupkati*	Elliptical to round	Medium	Light green	Few	White	Semi loose
Kanchannagar*	Pear shape	Medium	Green	Very few	White	Semi losse
Kazipayara*	Elliptical	Large	Yellowish green	Few	White	Crisp

* Commercial improved varieties

Table 2: *In vitro* and *in situ* reaction of local guava varieties inoculated with anthracnose causal pathogens

Pathogen	MOI	NFI	<i>In vitro</i>							
			Local Var. I		Local Var. II		Local Var. IV		Local Var. VII	
			PFI	ALS	PFI	ALS	PFI	ALS	PFI	ALS
<i>Botryodiplodia throbromae</i>	P	4	100	8.25	100	8.50	100	9.50	100	8.00
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-
<i>Pestalotiopsis psidii</i>	P	4	0	-	0	-	0	-	100	11.50
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-
<i>Colletotrichum gloeosporioides</i>	P	4	100	3.90	100	8.75	100	9.00	0	-
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-

Table 2: Continued

Pathogen	MOI	NFI	<i>In situ</i>							
			Local Var. I		Local Var. II		Local Var. IV		Local Var. VII	
			PFI	ALS	PFI	ALS	PFI	ALS	PFI	ALS
<i>Botryodiplodia throbromae</i>	P	4	100	9.50	100	8.5	100	11.00	100	12.00
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-
<i>Pestalotiopsis psidii</i>	P	4	0	-	0	-	0	-	100	12.00
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-
<i>Colletotrichum gloeosporioides</i>	P	4	100	10.5	100	12.5	100	8.00	0	-
	U	4	0	-	0	-	0	-	0	-
	C	4	0	-	0	-	0	-	0	-

Table 3: *In vitro* and *in situ* reaction of commercial improved guava varieties inoculated with anthracnose causal pathogens

Pathogen	MOI	NFI	<i>In vitro</i>					
			Sarupkati		Kazipayara		Kanchannagar	
			PFI	ALS	PFI	ALS	PFI	ALS
<i>Botryodiplodia throbromae</i>	P	4	100	9.50	100	10.75	100	8.75
	U	4	0	-	0	-	0	-
	C	4	0	-	0	-	0	-
<i>Pestalotiopsis psidii</i>	P	4	100	8.25	100	9.50	100	8.50
	U	4	0	-	0	-	0	-
	C	4	0	-	0	-	0	-
<i>Colletotrichum gloeosporioides</i>	P	4	100	9.10	100	9.75	100	8.20
	U	4	0	-	0	-	0	-
	C	4	0	-	0	-	0	-

Table 3: Continued

		<i>In vitro</i>							
		Sarupkati			Kazipayara			Kanchannagar	
Pathogen	MOI	NFI	PFI	ALS	PFI	ALS	PFI	ALS	
<i>Botryodiplodia throbromae</i>	P	4	100	10.25	100	11.50	100	1.50	
	U	4	0	-	0	-	0	-	
	C	4	0	-	0	-	0	-	
<i>Pestalotiopsis psidii</i>	P	4	100	9.50	100	11.25	100	9.25	
	U	4	0	-	0	-	0	-	
	C	4	0	-	0	-	0	-	
<i>Colletotrichum gloeosporioides</i>	P	4	100	10.10	100	11.50	100	8.75	
	U	4	0	-	0	-	0	-	
	C	4	0	-	0	-	0	-	

P: Pricked, U: Unpricked, C: Control (pricked and unpricked covered with wef control),

Mol: Methods of inoculation, NFI: Number of fruits infected, PFI: Per cent fruit infected, ALS: Average lesion size (mm)

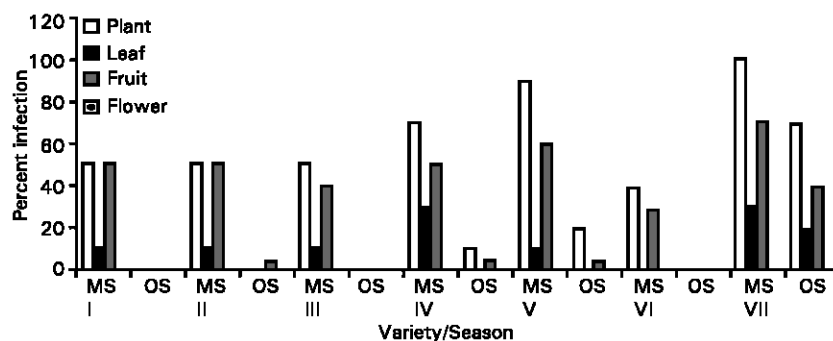


Fig. 1: Incidence of anthracnose on guava variety local at BAU-Campus during main and off seasons. (MS: Main season and OS: Off season)

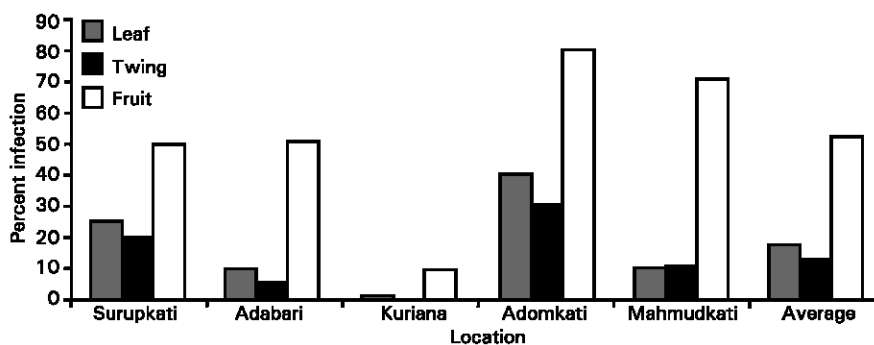


Fig. 2: Natural incidence of anthracnose on different plant parts of variety sarupkati at different locations during main crop season, 1990 in Pirozpur district

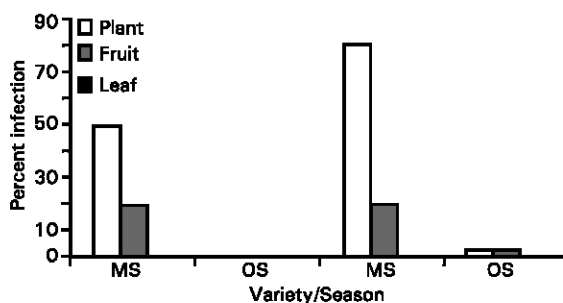


Fig. 3: Incidence of guava anthracnose in two commercial varieties during off (OS) and main seasons (MS) at Horticulture base, Kawatkhali in Mymensingh

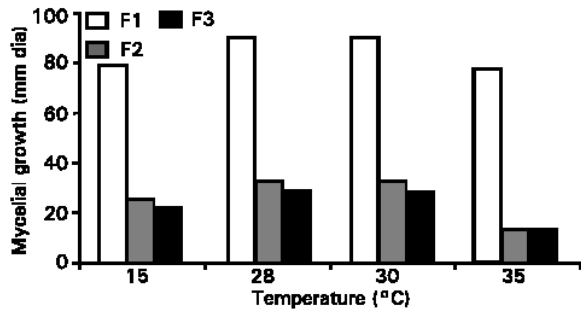


Fig. 4: Mycelial growth of *Botryodiplodia theobromae* (F1), *Pestalotia psidii* (F2) and *Colletotrichum gloeosporioides* (F3) After 24 h of incubation at different temperature

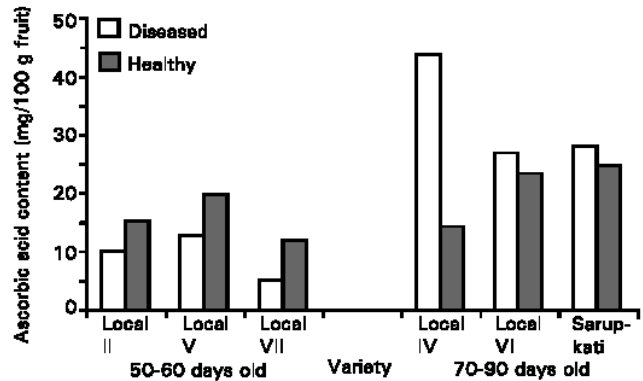


Fig. 6: Ascorbic acid content in both healthy and diseased fruits of some selected varieties at different age

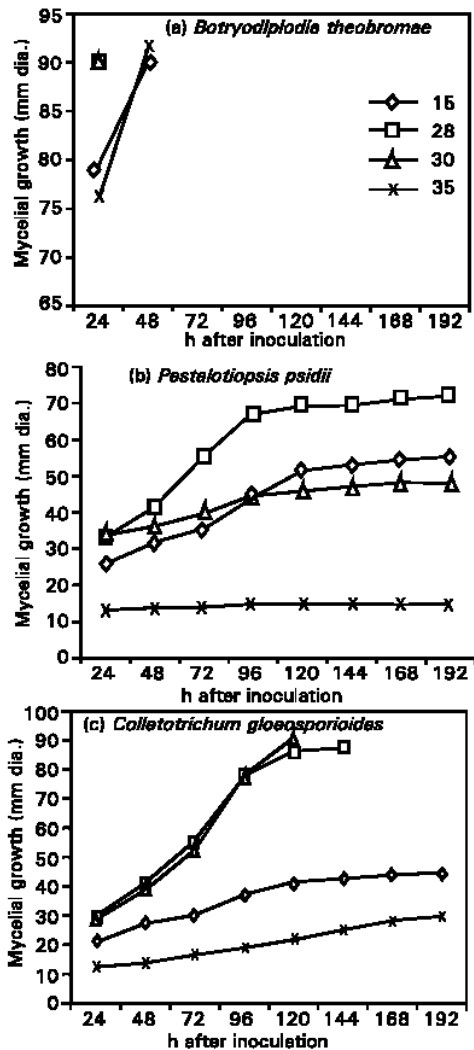


Fig. 5: Mycelial growth of *Botryodiplodia theobromae* (a), *Pestalotia psidii* (b) and *Colletotrichum gloeosporioides* © at different temperatures and incubation period

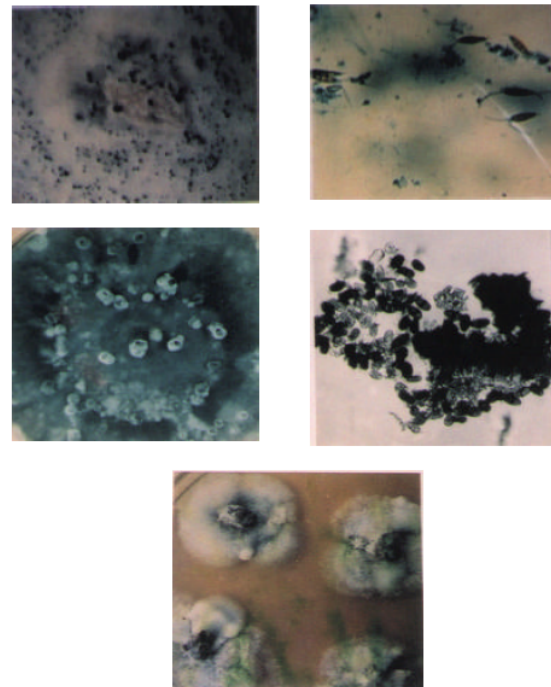


Plate 1: Identified causal organisms of guava anthracnose
 A. Culture (I) and Conidia (II) of *Pestalotia psidii*
 B. Culture (I) and Pycnidia with Pycnidiospores (II) of *Botryodiplodia theobromae*
 C. Culture of *Colletotrichum gloeosporioides*

Varietal characteristics: Both external and internal characteristics of guava fruits from local varieties revealed a variation in shape, size, color (skin and flesh), seed status and texture of flesh. The local cultivars were categorized into seven varieties I. e. Local I, Local II, Local III, Local IV, Local V, Local VI and Local VII considering the above mentioned characters (Table 1). Other

commercially important improved variety I. e. sarupkati, kazipayara and kanchannagar were also reported as those of local cultivars.

Isolation and identification of the causal organisms:

Inocula prepared from diseased fruits were transferred to potato dextrose agar media for isolation of the causal organism. Three fungi as *Pestalotiopsis psidii*, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were identified as the causes of guava fruit anthracnose (Plate 1).

Growth of the organisms: *Botryodiplodia theobromae* was the fast growing fungus followed by *Pestalotia psidii* and *Colletotrichum gloeosporioides* (Fig. 4). At low (15°C) and high (35°C) temperatures, *Botryodiplodia theobromae* grew a bit slowly when the fungus attained a growth of 7.6-7.9 cm after 24 h. However at 28 and 30°C temperature, the fungus covered the whole plate (9.0 cm) within 24 h (Fig. 5-a)

The growth of both *P. psidii* and *C. gloeosporioides* were slow at 15 and 35°C (5- b and c). At 15°C *P. psidii* attained a linear growth of maximum 5.5 cm and *C. gloeosporioides* showed 4.5 cm after 192 h of inoculation. Final observation on the growth of *P. psidii* and *C. gloeosporioides* was 1.5 and 3.0 cm at 35°C, respectively. Temperatures 28 and 28-30°C were found the most suitable for *P. psidii* (Fig. 5b) and *C. gloeosporioides* (Fig. 5c) respectively. However, growth of *C. gloeosporioides* was faster than the growth of *P. psidii* at all the temperatures except in low 15°C (Fig. 5b and c). Both lower and higher temperatures did not favour the growth of pathogens. All the pathogens grew well at 28-30°C with the exception of *P. psidii* at 30°C.

Evaluation of guava varieties

In vitro inoculation: Symptoms were developed in pricked inoculated fruits. Unpricked inoculated, control (both pricked and unpricked covered with wet cotton wool) and untreated fruits developed no symptoms. Local variety I, II, and IV inoculated with *B. theobromae* and *C. gloeosporioides* developed anthracnose symptoms and inoculation with *Pestalotiopsis psidii* was not successful. On the other hand, Local var. VII inoculated with *B. theobromae* and *P. psidii* developed symptoms but in case of *C. gloeosporioides* there were no symptoms. Pear shaped local varieties (I and II) developed smaller lesions than elliptical (var. IV) or round shaped local varieties (var. VII) (Table 2).

In situ inoculation: Symptoms developed were the same as *in vitro* inoculation to local var. II with three fungi.

Lesion size produced by *B. theobromae* in *in vitro* inoculation varied from 8.00-9.5 mm on four local varieties and lesions produced by *P. psidii* and *C. gloeosporioides* varied from 0.0-11.5 and 3.9-9.00 mm, respectively (Table 2). Lesion size produced in *in situ* inoculation by the three fungi was always larger than that in *in vitro* inoculation. Commercially improved variety sarupkathi, kazipayara and kanchannagar inoculated with *B. theobromae*, *P. psidii* and *C. gloeosporioides* developed anthracnose symptoms. Symptoms were developed only in pricked inoculated fruits but unpricked inoculated, control fruits did not develop any symptoms. Lesion size produced by *B. theobromae* varied from 8.75-10.75 mm while those produced by *P. psidii* varied from 8.25-9.5 mm and those produced by *C. gloeosporioides* varied from 8.2-9.75 mm on the above three commercial varieties (Table 3). Results were almost similar to those obtained in *in vitro* inoculation in case of *in situ* test.

Ascorbic acid: Immature fruits (50-60 days) showed that amount of ascorbic acid in tested varieties was less in diseased fruits than in healthy ones (Fig. 6). Ascorbic acid varied from 12.08-19.80 mg/100 g in disease free and 5.09-12.80 mg/100 g in diseased fruits. In comparatively older fruits (70-90 days), ascorbic acid was more in diseased fruits than the healthy ones.

Discussion

Forty to hundred percent fruits were found severely infected with anthracnose during main season and less or no infection in off-season. Tandon and Singh (1969) reported that anthracnose symptoms on the fruits were specially detected during rainy season (main season). High humidity and rainfall as well as high temperature enhance the disease incidence. Tandon and Singh observed that 96.1% rh and 30 and 35°C were the optimum temperature for disease spread on unripe and ripe fruits respectively. Kaushik *et al.* (1972) found that fruit infection and disease intensity increased at 35°C and 100% rh under natural conditions. In Bangladesh, the main guava season is hot and humid and the off-season is cool and dry. This explained the record of higher disease intensity during MS than in OS. This further explained the failure of fruit infection following the inoculation during OS. Earlier reports on survey on the prevalence of guava anthracnose in Perozpur, Sylhet, Mymensingh and Chittagong districts supports the results of the present study (Anonymous, 1985; Meah and Khan, 1987; Hossain and Meah, 1992). High prevalence of the disease has also been reported from India (Srivastova and Tandon, 1969; Kapoor and Tandon, 1970) and Nigeria (Adisa, 1985).

Fruit characteristics, specification of local guava cultivars

into seven varieties and their reaction to anthracnose causal agents revealed pathogen specific varietal source of disease resistance. Local varieties were inoculated with three fungi *B. theobromae*, *P. psidii* and *C. gloeosporioides*. Inoculations with *B. theobromae*, and *C. gloeosporioides* were successful where typical anthracnose symptoms were developed. With the exception of one local variety, inoculation with *P. psidii* did not develop symptoms to other local varieties but it produced lesion in all commercial varieties. Local varieties of pear shaped produced smaller lesions than round shaped ones. Similar results were obtained with pear shaped commercial variety kanchannagar where smaller lesions were developed than those on near-round sarupkati and kazipayara. Narashimhan (1939) reported that attempts to inoculate wounded and unwounded unripe guava fruits with spores of *P. psidii* and *Gloeosporium* sp. were not successful. In India various studies indicated that pear shaped guava and apple guava (light red fleshed) were less susceptible whereas variety safeda (white fleshed) was very susceptible (Srivastava and Tandon, 1969; Tandon and Singh 1969). Comparatively local varieties were less susceptible than commercial varieties. No variety was found resistant against all three anthracnose causal fungi. However, in a study Naresh Mehta *et al.* (1988) produced 146 guava hybrids, 40 of them showed resistant reaction to *Glomerella cingulata*.

Three fungi as *Botryodiplodia theobromae*, *Pestalotiopsis psidii* and *Colletotrichum gloeosporioides* were isolated from diseased fruits and their pathogenicity had been established. Hossain and Meah (1992) also reported the above fungi as the causal organisms. Anthracnose of guava caused by *C. gloeosporioides* (Pathak, 1986) scab or canker caused by *P. psidii* (Kaushik *et al.*, 1972; Pathak, 1986) and association of *C. gloeosporioides* or *Gloeosporium psidii* with anthracnose of guava fruits (Gupta *et al.*, 1973) have been reported. Both of the fungi *Glomerella psidii* and *Pestalotia psidii* have been reported to be isolated from young green and mature guava fruits and leaf spots (Venkatakrishniah, 1954). Isolation of *G. psidii* from infected fruits, twigs, and leaves of guava has also been reported (Tandon and Singh, 1969).

The growth of the causal organisms was observed under different temperatures. *Botryodiplodia* sp. was the most fast growing fungus at all temperatures within the identified fungi. Temperatures 28 and 28-30°C were found the most suited for growth of *P. psidii* and *C. gloeosporioides*, respectively. Srivastava and Tandon (1969) reported that the fungi *C. gloeosporioides* and *P. psidii* could thrive between 15 and 35°C. But the optimum

temperature for growth was 25°C. The growth of both *P. psidii* and *C. gloeosporioides* were slow at 15 and 35°C as observed in the present study as well. In an attempt Tandon and Singh (1969) reported the minimum temperatures for disease spread on ripe and unripe fruit were 10 and 15°C, respectively where the maximum temperature for both the cases was 35°C.

Immature diseased fruits contained lower amount of ascorbic acid than the healthy ones. The decrease in ascorbic acid content in guava with the infection of pathogen has been reported by Kapoor (1982). There are further reports of decline in ascorbic acid content of pears due to infection by *Aspergillus flavus* (Sinha and Singh, 1984). These findings are in agreement with the present study. Again, Anwar *et al.* (1986) found that powdery mildew resistant varieties of maize and sorghum contained higher ascorbic acid than the susceptible varieties. There are reports that an increase in ascorbic acid (up to 10 ppm) containing media gave the highest dry weight of mycelium and good sporulation of *C. gloeosporioides* causing guava fruit rot (Shukla, 1972). He also reported that at higher concentration of ascorbic acid the growth of mycelium was declined. This study indicated that infected mature fruits contained more amount of ascorbic acid than the healthy ones. Therefore more investigation will be needed to know the relationship of ascorbic acid content and the disease development.

Based on the above discussion it might be concluded that guava anthracnose is present all over the Bangladesh and occurs with higher disease intensity during main season than in off-season. Guava fruit anthracnose caused by all three identified fungi in Bangladesh. Against all three pathogens no varieties found resistant in this study but pathogen specific few resistant local cultivars were identified. Susceptibility to anthracnose may be attributed to the shape of varieties and ascorbic acid contents of fruits. Further studies are required to establish a relation between shape, ascorbic acid contents, other anti pathogen chemicals and anthracnose infection of guava fruits.

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