



Leaf blight and fruit rot disease of brinjal caused by *Diaporthe vexans* (*Phomopsis vexans*) in six agro-ecological regions of South West India

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Abstract

Diaporthe vexans associated with leaf blight and fruit rot disease of brinjal is a serious fungal pathogen causing a significant economic loss in terms of production. Leaf blight and fruit rot disease severity across six agro-ecological zones of Karnataka (India) was studied and the isolation frequency of *D. vexans* from the six regions was determined. *D. vexans* isolates were grown on potato dextrose agar medium and identified based on morphological and cultural characteristics. High severity of leaf blight disease was recorded in northern transition zone (NTZ: 10.6–25.3%) followed by central dry zone (CDZ: 10–17%) and southern dry zone (SDZ: 8.3–18%). The maximum severity of fruit rot disease was in CDZ (29–39%) followed by SDZ (22.3–62%) and NTZ (21–33.3%). The frequency of occurrence of *D. vexans* ranged from 90–100% in all the zones studied and the isolates were all similar in morphology and cultural characteristics. The study concluded that *D. vexans* is a serious constraint to brinjal production in six brinjal growing regions of southwest India.

Key words – brinjal – disease severity – identification – morpho-cultural– *Phomopsis vexans* – *Solanum melongena*

Introduction

Species of *Diaporthe* and their asexual morphs (*Phomopsis* species) have broad host ranges and are widely distributed as plant parasites, endophytes and saprobes (Carroll 1986, Boddy & Griffith 1989, Murali et al. 2006, Botella & Diez 2011, Udayanga et al. 2011, Gomes et al. 2013). *Diaporthe* species are responsible for some important crop diseases worldwide including root rots, fruit rots, dieback, cankers, leaf spots, blights, decay and wilt (Mostert et al. 2001, Santos et al. 2011, Thompson et al. 2011). Endophytic *Diaporthe* species can deter herbivory, have lingo-cellulolytic activities, or act as bio-herbicides. *Diaporthe vexans* (asexual morph *Phomopsis vexans*) on brinjal, causes severe damage to the brinjal crop in different regions of the world.

Brinjal (*Solanum melongena* L., *Solanaceae*) is an agronomically important non-tuberous crop native to southern India, and widely grown in America, Europe and Asia (Sekara et al. 2007). It is one of the most important vegetable crops in India (Rashid 1976, Zeven & Zhukovsky 1975, Sekara et al. 2007).

Fruit rot and leaf blight disease caused by *Diaporthe vexans* is of major concern in brinjal producing areas of India as it reduces yield and marketable value of the crop by 20–30% (Das 1998, Khan 1999). In Karnataka, leaf blight and fruit rot disease is a major limiting factor for brinjal crop production in recent years. Hence, the present study was conducted to determine disease severity of *Diaporthe* in six major agro-ecological regions of south west India, and to characterize *D. vexans* by morpho-cultural methods.

Materials & Methods

Study area

The study was carried out in six agro ecological zones of Karnataka, India for three years (2011–2013) (Fig. 1). The survey area covered approximately 1200–1350 km² each year. The classification of the six agro-ecological zones is based on data provided by Indian Council of Agricultural Research, New Delhi and the Department of Agriculture, Government of Karnataka State.

Severity assessment

The severity of leaf blight and fruit rot disease was assessed by categorizing all the infected samples into different grades of infection using the grading scale 0–5 (McKinney 1923, Hossain et al. 2010). During 2011, 2012 and 2013, a total of 335, 370 and 342 fields, respectively, were surveyed in the six agro-ecological zones (AEZ). The disease severity in terms of per cent disease index (PDI) was calculated as,

$$\text{PDI} = (\text{Sum of numerical values} / \text{total number of leaves observed} \times \text{maximum grading}) \times 100.$$

Numerical values obtained by multiplying the number of infected leaves/fruits with their respective grades. Based on PDI, each locality was identified with the following grades. I - 0 PDI: free from the disease; II - 0.1–5.0 PDI: poorly affected; III - 5.1–20.0 PDI: moderately affected; IV - 20.1–50.0 PDI: severely affected; and V - >50.1 PDI: very seriously affected by the disease.

Morphological and cultural identification of *D. vexans*

Representative leaf blight and fruit rot samples collected from six AEZ were brought to the laboratory for isolation of the associated fungal pathogens (Dhingra & Sinclair 1985). Samples were cut into small pieces (5 mm) and surface sterilized with 2% sodium hypochlorite solution for 2–3 min. followed by three washes with sterile distilled water. Samples were blotter dried and placed on potato dextrose agar (PDA) medium and incubated for 7 days at 28±2°C. Fungal colonies expressed after 7 days of incubation were identified based on micro-morphology of vegetative structures, pycnidia and conidia. The fungal isolates were sub-cultured and stored at 4°C for further studies. The isolation frequency of *D. vexans* was determined by the number of samples having *P. vexans* and the total number of samples plated using the formula:

$$\text{Isolation frequency } (D. \textit{vexans}) = (\text{Number of samples having } D. \textit{vexans} / \text{Total number of samples plated}) \times 100.$$

Statistical analysis

Data on severity from each agro-ecological zone were subjected arithmetic mean and SD calculated using the mean values for each study area. Dimensions were made for 50 conidia and the mean value is presented.

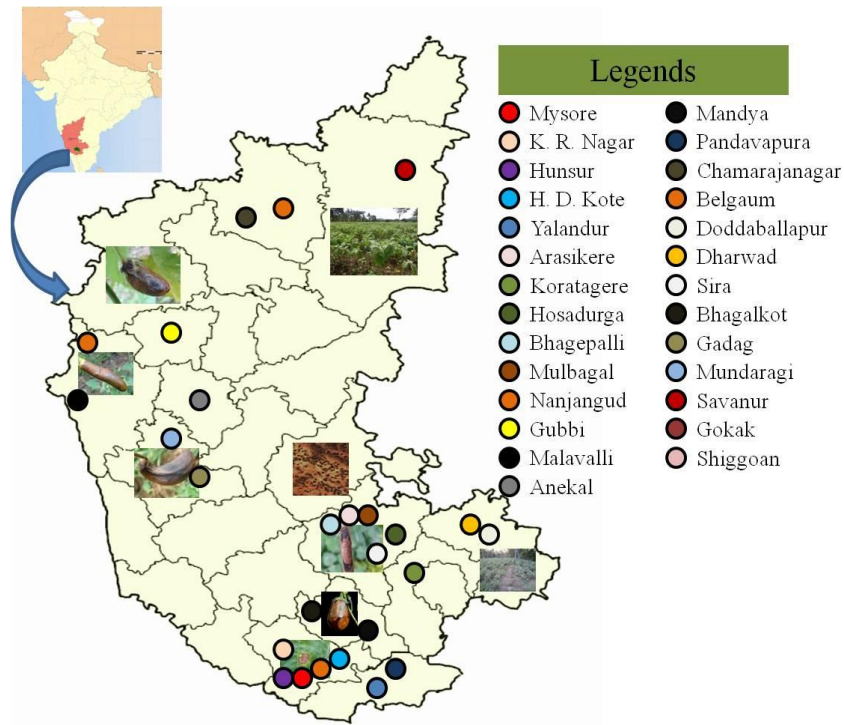


Fig 1 – Map of Karnataka showing the study localities visited to assess leaf blight and fruit rot disease severity in brinjal.

Results

The severity of leaf blight and fruit rot disease was determined (Figs 2, 3). The disease was prevalent (100%) in all the studied regions. High severity of leaf blight (15.2–53.7%) and fruit rot (41–78%) was recorded in SDZ followed by NTZ (12.1–17.2% -leaf blight disease). The lowest severity of leaf blight (3.8–12.8 and 8.4–34.2%) and fruit rot (24–62 and 3.1–70) disease was observed in NTZ and STZ, respectively.

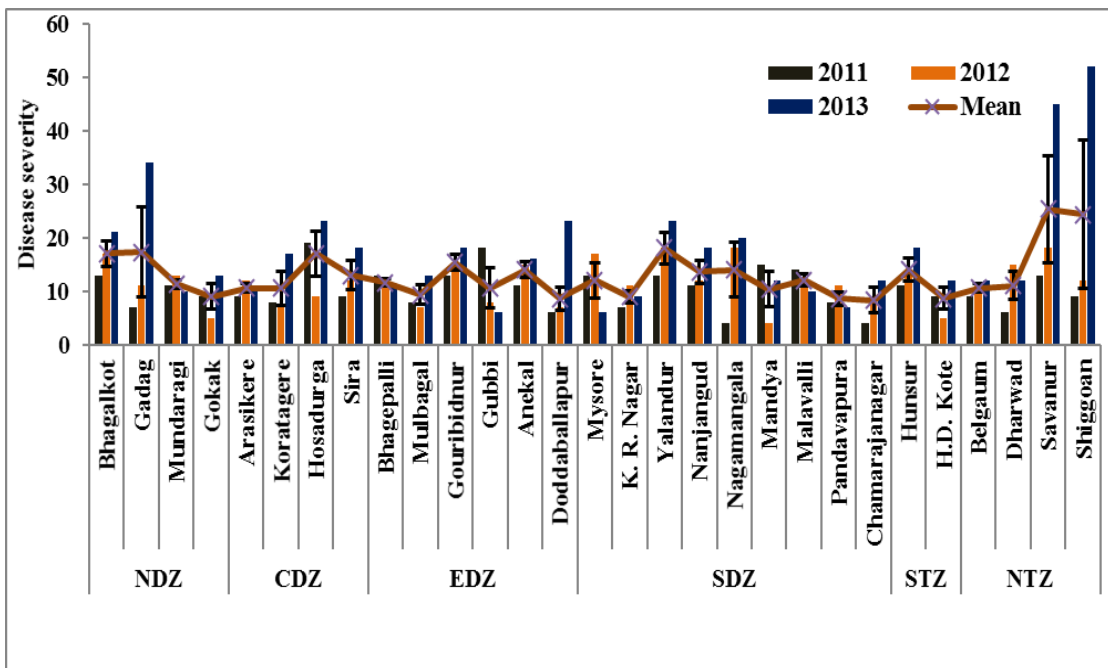


Fig 2 – Severity of leaf blight disease on brinjal from 2011–2013 in six agro-ecological regions of South West India. Bars represent the standard deviation for the mean values for each study area.

In many zones, the incidence of fruit rot was very high when compared to leaf blight and it was responsible for severe fruit loss. The severity of leaf blight and fruit rot diseases varied from zone to zone and the severity differed in each year. Though there was difference between the severity of leaf blight and fruit rot disease, the leaf blight severity influenced the fruit rot severity as the inoculum load builds up as the crop reaches maturity.

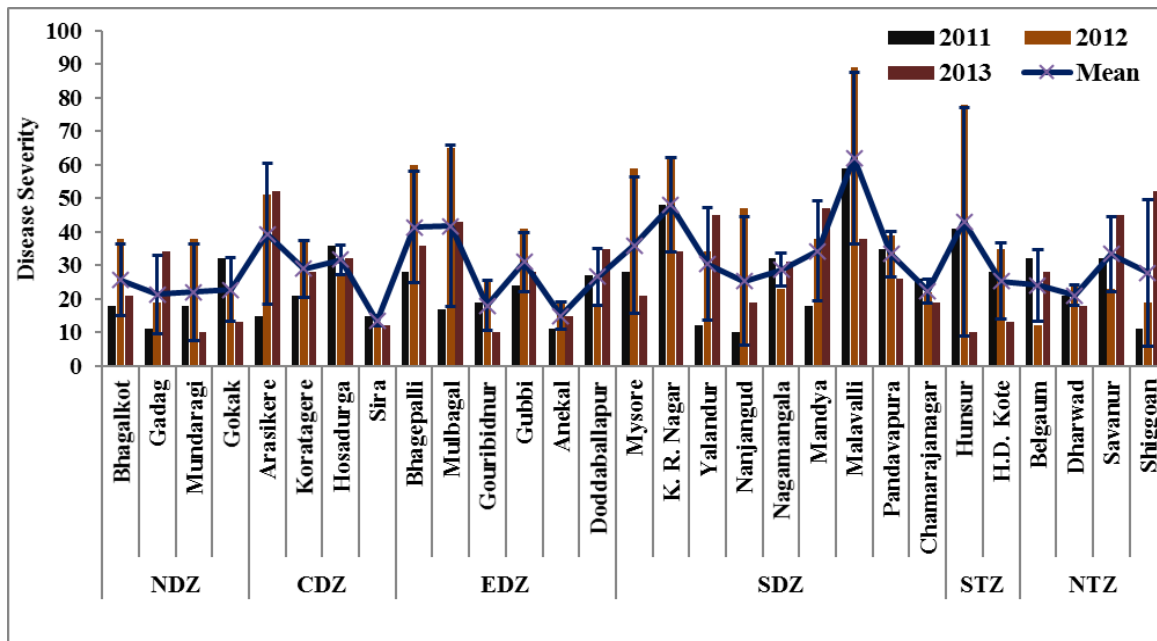


Fig 3 – Severity of fruit rot disease on brinjal from 2011–2013 in six agro-ecological regions of South West India. Bars represent the standard deviation for the mean values for each study area.

A total of 1007 samples of leaf blight, fruit rot and stem blight samples were collected across six AEZ; 921 samples were infected by *D. vexans* as evident on PDA isolation. The number of samples and isolation frequency of *D. vexans* is presented in Table 1. The isolation frequency varied between different agro-ecological zones. Highest frequency of *D. vexans* was recorded from NDZ for leaf blight (94.73%) and SDZ for fruit rot (95.83%) disease. Stem blight disease was observed in EDZ, SDZ and STZ with 100%, 89.47% and 100% (mean isolation frequency 95.54%) isolation frequency, respectively.

Table 1 Isolation frequency of *Diaporthe vexans* from six agro-ecological regions

Sl. No	Agro-Ecological Zone	Number of Samples collected*			Isolation Frequency		
		LB	FR	SB	LB	FR	SB
1	Northern Dry Zone (NDZ)	95	78	NF**	94.73	76.92	-
2	Central Dry Zone (CDZ)	112	64	-	85.71	93.75	-
3	Eastern Dry Zone (EDZ)	40	52	14	100	92.30	100
4	Southern Dry Zone (SDZ)	118	96	38	91.52	95.83	89.47
5	Southern Transition Zone (STZ)	64	46	34	100	100	100
6	Northern Transition Zone (NTZ)	69	87	-	79.71	91.95	-
	Overall	498	423	86	90.96	91.25	95.54

Note: LB-Leaf Blight; FR-Fruit Rot; SB-Stem Blight; *Value for total number of samples indicates the cumulative value for samples collected in survey period (2011–13); **NF-Not found

Fruit rot symptoms appeared first as a minute sunken greyish spots with a brownish halo, which later enlarge and coalesce to form concentric rings of yellow and brown necrotic zones. The rotten areas increased in size and with the appearance of conidiomata concentric zones (Fig. 4).



Fig 4 – Typical leaf blight and fruit rot disease symptoms on brinjal. A–C, Leaf blight symptoms. D, Stem blight symptom. E, F, Fruit rot disease. G–J, Pycnidia on necrotic leaves. K–M, Alpha conidia of *D. vexans* observed under compound microscope (Scale bars = 20 μ m).

Colonies of *D. vexans* isolates on PDA were white to pale pink with wavy margins. Pycnidia were submerged and formed all over the surface of the mycelium (Fig. 5). The number and size of pycnidia varied from isolate to isolate. The mycelium was hyaline and septate; the conidiophores (phialides) within the pycnidium were hyaline, simple, or septate and arose from the innermost layer of cells lining the pycnidial wall. Two types of conidia (alpha and beta) were observed. Alpha conidia were hyaline, single celled, biguttulate and subcylindrical ($4.1\text{--}6.5 \times 1.2\text{--}1.9 \mu\text{m}$). Beta conidia were filiform, curved, hyaline and septate ($6.2\text{--}7.6 \times 0.5\text{--}0.8 \mu\text{m}$).

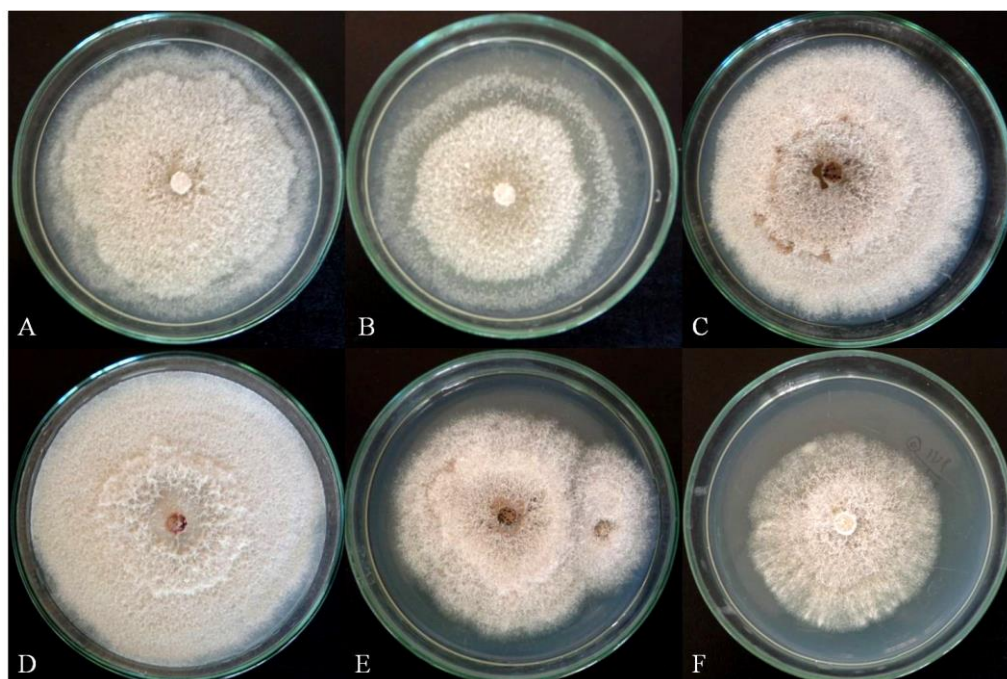


Fig 5 – Colony morphology of *Diaporthe vexans* (pure cultures on PDA) isolated from A, leaf blight disease; B, fruit rot disease; C, stem blight disease; D, infected seed; E & F, damping-off disease.

Discussion

The brinjal crop is susceptible to both biotic and abiotic stresses during different stages of crop production. Among the biotic agents, fungal pathogens are responsible for significant reduction in crop yield. The most important and widespread fungal diseases are leaf blight and fruit rot (*Diaporthe vexans*), leaf spot (*Alternaria melongenae* and *Cercospora melongenae*), damping-off (*Pythium aphanidermatum*), wilt (*Verticillium dahliae*) and root rot (*Sclerotinia sclerotiorum*) (Shivaprakasam & Soumini Rajgopalan 1974, Iqbal et al. 2003).

A field survey conducted in UP (Mishra & Mishra 2012), Karnataka (Jayaramaiah et al. 2013) and Allahabad (Singh et al. 2014) reported the occurrence of *Diaporthe vexans* but quantitative data is lacking. There are no reports assessing the severity of leaf blight disease. The incidence and severity of disease is greatly influenced by the weather conditions (Seem 1984) and cultural practices (sprinkling irrigation) adapted during crop cultivation. The splash dispersal of soil or fruit-borne conidia might have caused new infections resulting in high disease incidence in many zones.

Diaporthe vexans produces different disease symptoms such as damping-off, leaf blight, fruit rot and stem blight on brinjal crop. Damping-off of brinjal includes the development of girdling signs at the base of the stem and soil interface. The affected plants topple and die due to rotting of root system (Singh 1992). Pycnidia were noticed on the girdle and the death of whole seedling was observed. Leaves emerging from infected seedlings also showed leaf spot disease due to early infection. Leaf blight symptoms includes the development of tan or brown oval necrotic zones, which later become irregular and coalesce. Lesions on the petiole or the lower part of the midrib resulted in early senescence, and the blight affected areas produced numerous black pycnidia (Harter 1914, Jayaramaiah et al. 2013). At first, the lesions were small, more or less circular, and buff to olive, later becoming cinnamon buff, with an irregular blackish margins. Rotting of fruit was noticed during transit and even after harvest (Sherf & MacNab 1986). On stems and aerial branches, elongated, blackish brown lesions were observed. The affected plants produced small leaves and the axillary buds were often killed. When stem girdling was severe, the shoots showed wilting followed by death of infected seedlings. Affected plants were toppled by the wind (Edgerton & Moreland 1921, Pawar & Patel 1957, Sherf & MacNab 1986). Pycnidia were seen on

lesions on young stems, but rarely on older ones (Harter 1914). Pycnidia on fruit were bigger than those produced on stems and leaves (Harter 1914). In severe infection, the whole fruit was mummified (Pawar & Patel 1957).

In conclusion, the study indicated that *D. vexans* is distributed in all the six agro-ecological zones of southwest India and considerable crop loss was recorded in all zones. *D. vexans* was the predominant fungal pathogen associated with leaf blight and fruit rot disease. As the disease severity increased year after year in major brinjal growing regions, there is a need for screening and identifying disease resistant cultivars against leaf blight and fruit rot disease. This will be the long term strategy to prevent crop losses due to *D. vexans* infection.

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