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Leaf blight of *Syzygium cumini* and its management *in vitro*

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Abstract. A *Pestalotiopsis* sp. identified as the major pathogen causing leaf blight disease of *Syzygium cumini* was isolated from naturally infected leaf samples of *S. cumini* collected from forest nurseries of the Mysore district, India. The fungus was pathogenic on 4-month-old seedlings, which exhibited leaf blight symptoms within 15 days of inoculation. The effects of five systemic and two contact fungicides were evaluated against the pathogen *in vitro* using the poison food technique. Among the seven fungicides studied *in vitro* only two systemic fungicides namely, Bavistin and Roko were proven to be effective against *Pestalotiopsis* sp. at concentrations of 50, 100 and 150 mg/L; these fungicides showed 100% growth inhibition and nil fungal growth. The effectiveness of systemic fungicides was higher than that of contact fungicides. Thus, the present study recommends the use of Bavistin and Roko at a minimal concentration of 50 mg/L for maximum inhibition of *Pestalotiopsis* sp.

Introduction

Jambolan (*Syzygium cumini* L. Skeel) is a fast-growing, medicinally important plant of the family Myrtaceae and is one of the most common plants grown in forest nurseries of the Mysore district. The leaves are antibacterial and are used locally for strengthening teeth and gums. The fruits and seeds are sweet, acrid, sour, tonic and cooling and are used medicinally in diabetes, diarrhoea and ringworm (Prajapati *et al*. 2003). The bark is astringent, sweet, sour, diuretic, digestive and antihelminthic. The juice of the ripe fruit or a decoction of the fruit or jambolan vinegar may be administered in India in cases of enlargement of the spleen, chronic diarrhoea and urine retention. Water-diluted juice is used as a gargle for sore throats and as a lotion for ringworm of the scalp (www.alternative-healthguide.com, verified 2 August 2007).

Pestalotiopsis has always been considered to be a weak parasite and of minor importance in different crops and is being managed by manipulating existing cultural practices (Bilgrami *et al*. 1979). This pathogen has been recorded on a wide variety of hosts, mostly on their leaves, fruits and in the rhizosphere (Bilgrami *et al*. 1979). Early studies indicated that *Pestalotiopsis* sp. is ubiquitous in distribution, occurring on wide range of substrata. Many of them are saprobes (Wu *et al*. 1982), while others are pathogenic on a wide range of hosts causing various diseases including stem canker, necrotic lesions on leaf, seed and root rots in different hosts (Madar *et al*. 1991; Yuan 1996; Yuan and Mohammed 1999; Vujanovic *et al*. 2000; Dhingra *et al*. 2002, 2003). In the present study, a *Pestalotiopsis* sp. was identified as the major pathogen causing leaf blight on seedlings of *S. cumini*. Therefore, studies were conducted to evaluate the response of seven fungicides under *in vitro* conditions.

Methods

Isolation of the pathogen

Leaves of *S. cumini* with leaf blight symptoms (Fig. 1) were cut into 1 cm^2 pieces and surface sterilised for 5 min using a 2% sodium hypochlorite solution followed by washing three times with distilled water. The leaf pieces were blotter-dried and placed on 2–3 layers of moistened blotters in Petri plates. The plates were incubated under 12 h/12 h cycles of lightness and darkness for 7 days. On 8th day the plates were screened for the pathogen.

Test for pathogenicity

A spore suspension of the leaf blight pathogen of *S. cumini* was prepared using a 7-day-old actively growing culture that was crushed in sterile distilled water using a pestle and mortar. The spore suspension was adjusted to 10^8 spores/mL using

Fig. 1. *Syzygium cumini* seedling with leaf blight symptoms.

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a haemocytometer and was then sprayed onto 4-month-old seedlings of *S. cumini* and covered with polythene covers to avoid secondary contamination. The polycovers containing *S. cumini* seedlings were watered daily to maintain humidity and observed regularly for symptoms.

In vitro *management*

Five systemic (Bavistin, Calixin, Contaf Plus, Roko and Tilt) and two contact (Blitox and Indofil M45) fungicides were tested at three different concentrations of 50, 100 and 150 mg/L for their efficacy against this leaf blight pathogen of *S. cumini* using the poison food technique (Dhingra and Sinclair 1985). Different concentrations of fungicides were prepared by dissolving the requisite quantity of each fungicide in warm potato dextrose agar before autoclaving. The autoclaved media were poured into Petri plates and allowed to cool. Actively growing 7-day-old culture of *Pestalotiopsis* sp. was cut into 0.4-cm diameter discs using a cork borer and was placed at the centre of each treatment. Each treatment was maintained in triplicate. Media without fungicide served as a control. The plates were incubated under 12 h/12 h cycles of lightness and darkness for 7 days. On the 8th day the radial growth of the mycelial colony was recorded and the percentage growth inhibition was calculated using the formula,

$$I = 100 \times (C - T)/C$$

where I is percentage inhibition, C is growth of fungus in the control and T is growth of fungus in the treatment.

The experiment was repeated three times and the data was analysed statistically by analysis of variance and the means were compared by Duncan's Multiple Range Test ($P < 0.05$).

Results

Isolation of pathogen

The pathogen was identified as a *Pestalotiopsis* sp. based on its morphological and conidial characters. Sooty black curls of conidia are the characteristic feature of the pathogen (Fig. 2*a*). Conidia have 3–5 septae, the middle cell is broad, the apical cell hyaline with three branched filiform appendages known as setulae. The basal cell is hyaline with a stipe (Fig. 2*b*).

Test for pathogenicity

Initial symptoms occurred after 30 days and became prominent after 2 months (Fig. 3). The leaves with blight symptoms were surface sterilised using a 2% sodium hypochlorite solution and subjected to the standard blotter method and the pathogen was reisolated.

In vitro *management*

Effect of fungicides against *Pestalotiopsis* sp. significantly differed from the control in response of radial growth irrespective of concentrations at 1% level of significance and percentage growth inhibition (Table 1).

Nil growth was recorded in all the three concentration of Bavistin (Carbendazim 50% WP) (Fig. 4) and Roko (Thiophanate methyl 70% WP) (Fig. 5) and 150 mg/L Calixin (Tridemorph 80% EC) (Fig. 6). Maximum growth of mycelial colony was recorded in control followed by all the three concentrations of Blitox (Copperoxychlorite 50% WP) (Fig. 7) followed by 100 mg/L Indofil M45 (Mancozeb 75% WP).

Maximum growth inhibition of 100% was found in case of all the three concentrations of Bavistin (Carbendazim 50% WP) and Roko (Thiophanate methyl 70% WP) and 150 mg/L Calixin (Tridemorph 80% EC). Minimum growth inhibition was found in all the three concentrations of Biltox (Copper oxychlorite 50% WP).

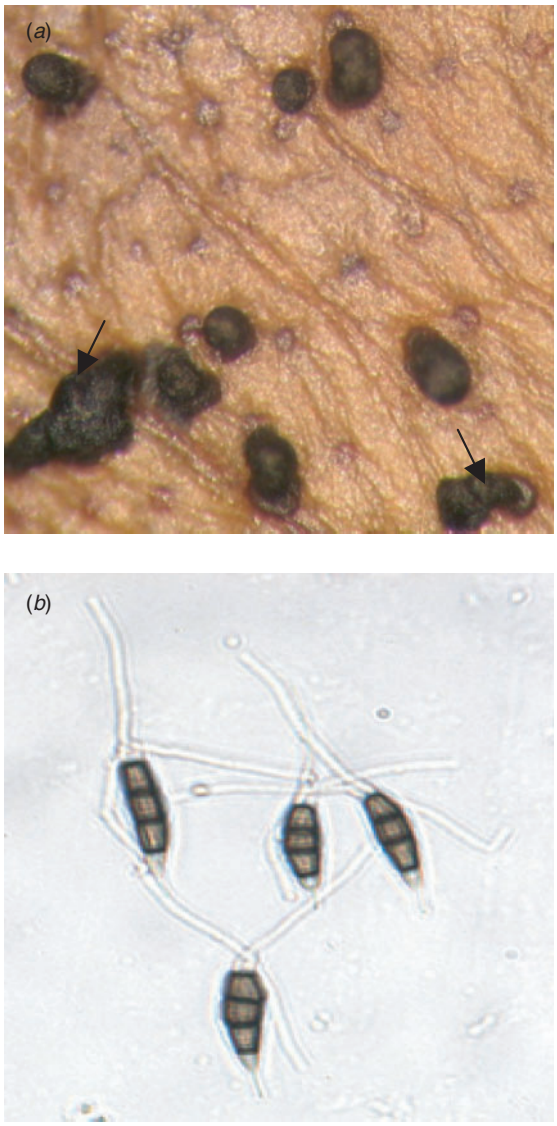

Fig. 2. (*a*) Sooty curls of *Pestalotiopsis* conidia. (*b*) Conidia of *Pestalotiopsis* sp.

Discussion

In this trial, the performance of systemic fungicides was better compared with contact fungicides against *Pestalotiopsis* sp.

Fig. 3. Seedling showing leaf blight symptoms, confirming the pathogenicity test.

Table 1. Effect of different concentrations of fungicides on mycelial growth of *Pestalotiopsis*

Data are the mean of three replicates for each concentration. Within columns, values followed by different letters are significantly different ($P < 0.05$)

Treatments	Concentration (mg/L)	*Pestalotiopsis* Fungal growth (cm) ± s.e.	*Pestalotiopsis* Growth inhibition (%) ± s.e.
Bavistin	50	0.00 ± 0.000a	100.00 ± 0.000g
	100	0.00 ± 0.000a	100.00 ± 0.000g
	150	0.00 ± 0.000a	100.00 ± 0.000g
Blitox	50	8.08 ± 0.321f	3.84 ± 5.112a
	100	8.22 ± 0.221f	2.07 ± 3.274a
	150	7.99 ± 0.005f	4.89 ± 1.019a
Calixin	50	0.91 ± 0.007a,b	89.18 ± 1.602f
	100	0.85 ± 0.001b	89.85 ± 0.119f
	150	0.00 ± 0.293a	100.00 ± 0.748g
ContafPlus	50	2.95 ± 0.522d	64.85 ± 4.725c
	100	1.48 ± 0.255c	82.45 ± 9.896e
	150	2.41 ± 0.153d	71.28 ± 4.398d
Indofil M45	50	6.06 ± 0.005e	28.05 ± 2.788b
	100	6.41 ± 0.008e	23.81 ± 0.817b
	150	6.13 ± 0.000e	27.37 ± 1.648b
Roko	50	0.00 ± 0.000a	100.00 ± 0.000g
	100	0.00 ± 0.000a	100.00 ± 0.000g
	150	0.00 ± 0.000a	100.00 ± 0.000g
Tilt	50	0.59 ± 0.173a,b	92.74 ± 2.658f
	100	0.53 ± 0.329a,b	93.65 ± 6.103f
	150	0.61 ± 0.233a,b	92.74 ± 4.178f
Control		8.40 ± 0.009f	

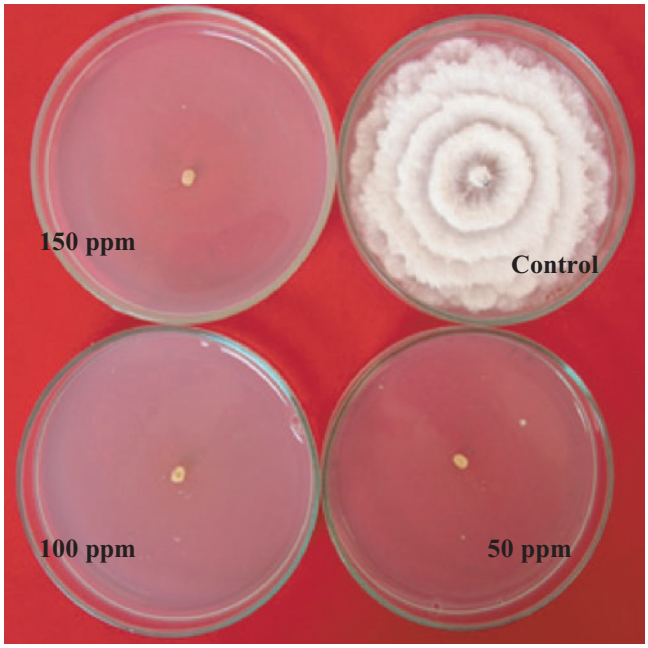

Fig. 4. Bavistin (Carbendazim 50% WP) treatment showing 100% growth inhibition in all the three concentrations.

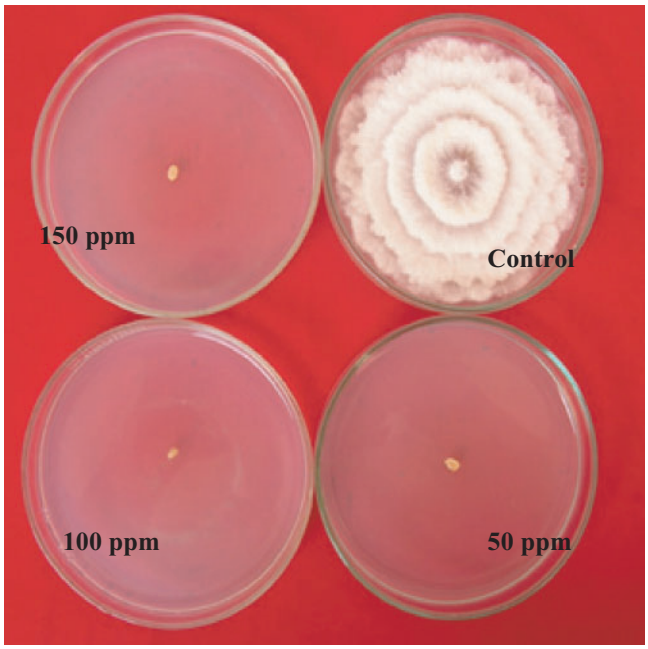

Fig. 5. Roko (Thiophanate methyl 70% WP) treatment showing 100% growth inhibition in all the three concentrations.

Harsh *et al*. (1987) reported the effectiveness of Bavistin at 0.1% concentration and Dithane M45 at 0.3% concentration against *Pestalotiopsis versicolor*, which causes foliar disease in *Diospyros melanoxylon* Roxb. Various workers have reported that Bavistin (Carbendazim), Tilt 250 EC, Cupravit and Dithane M45 (Mancozeb) performed best against *Pestalotia palmarum*

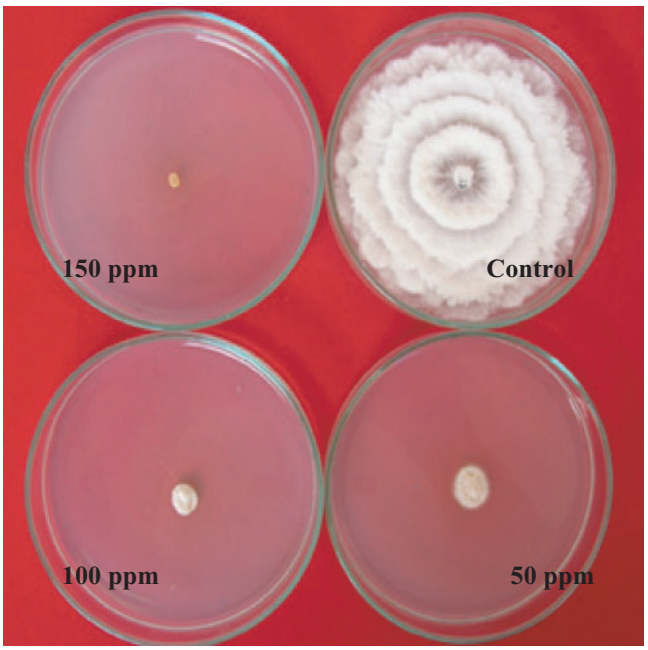

Fig. 6. Calixin (Tridemorph 80% EC) treatment showing 100% growth inhibition in the 150 mg/L concentration.

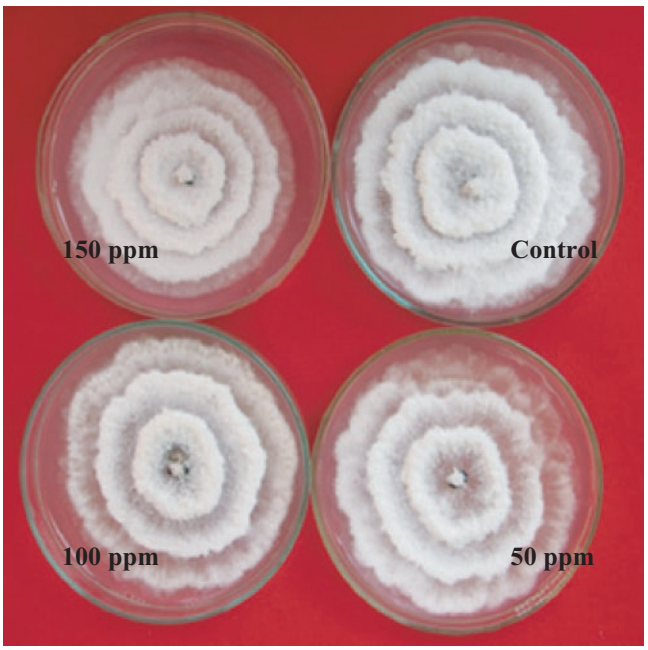

Fig. 7. Blitox (Copperoxychlorite 50% WP) treatment showing nil growth inhibition in all the three concentrations.

in vitro (Kundalkar *et al*. 1991; Joshi and Raut 1992; Selvan *et al*. 1993; Saw and Raut 1995; Khalequzzaman *et al*. 1998; Islam 2001). Complete inhibition of *Pestalotiopsis mangiferae* colony growth at the lowest concentration (0.11%) of Carbendazim was reported by Pandey *et al*. (2006). Even in the present study, Bavistin at all three concentrations (50, 100 and 150 mg/L) has been proved to be one of the most effective fungicides for causing complete inhibition of *Pestalotiopsis* sp. In contrast to the above reports, all the three concentrations of Indofil M45 (Mancozeb 75% WP) were less effective against *Pestalotiopsis* in the present study. Similarly, the performance of Dithane M45 against *Pestalotia palmarum*, the causal agent of leaf spot of Betelnut, was poor under *in vitro* tests (Islam *et al*. 2004). Our study suggests the use of minimal concentrations of fungicides for maximum inhibition of the fungus *Pestalotiopsis* sp. under *in vitro* condition.

The *Pestalotiopsis* sp. identified and isolated from the naturally infected leaf samples of *S. cumini* was pathogenic. Bavistin and Roko were the effective fungicides against *Pestalotiopsis* of *S. cumini* compared with all the systemic and contact fungicides studied. Therefore, Bavistin and Roko may be recommended in field trail for management of the disease caused by *Pestalotiopsis* sp.

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