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Antifungal activity of plant extracts against *Pestalotiopsis palmarum* causing leaf blight disease of coconut

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Coconut, in spite of its hardy nature, is affected by a number of diseases, which not only reduce yield but also kill the palms. Root (wilt), bud rot, basal stem rot, stem bleeding and grey leaf blight diseases are the major diseases affecting coconut causing heavy losses in India. Grey leaf blight disease caused by *Pestalotiopsis palmarum* is of common occurrence in all coconut growing areas throughout the world. The disease causes serious damage in nursery and in adult palms. The palms affected with grey blight flowered relatively late than healthy ones (Abad *et al*., 1975). The leaf blight incidence reduce the coconut yield to the extent of 10-24 per cent (Karthikeyan and Bhaskaran, 1999)

Adult coconut palms of 20-40 years of age were found highly susceptible to the leaf blight disease. The disease symptoms appear first as minute yellow spots with a grey brown margin on the outer whorl of leaves. The spots are oval in shape measuring 1 to 5 cm long (Menon and Pandalai, 1958). Gradually, the centre of the spots becomes grayish white while the brown colour of the margin deepens. Many spots coalesce to form large irregular necrotic patches. In advanced stage of infection it gives a blighted appearance (Nambiar, 1994).

Das and Mahanta (1985) reported the inhibition of *P. palmarum* under *in vitro* and *in vivo* conditions by spraying Carbendazim. Root feeding of Thiophanate-methyl or Carbendazim or Tridemorph at 2 per cent concentration reduced the severity of leaf blight disease of coconut in the field and increased nut yield. (Kathikeyan and Bhaskaran, 1998). Khalequzzaman *et al*. (1998) reported that Carbendazim (0.1%) was effective fungicide against grey leaf spot pathogen.

However, continuous use of specific systemic fungicides is not recommended, because, it has been found that systemic fungicides, when incorporated into the plant through stem and root, can leave residues in copra, nut-water and oil and also there could be the danger of pathogen acquiring resistance against these chemicals. Under these circumstances, environment friendly and less expensive methods like the use of plant extracts have great relevance. This led to the present investigation of screening plants for antifungal activity against *P. palmarum* causing grey leaf blight disease of coconut with the ultimate aim of developing an eco-friendly formulation for disease management.

The aqueous extracts of 10 plants were tested for their antifungal property against *P. palmarum* by poison food technique (Bhaskaran *et al*., 1988). Fresh plant parts were collected and washed with tap water followed by sterile water. The plant parts selected were mainly leaves except *Allium sativum*, in which bulbs were selected. Plant parts (100 g) were extracted with 100 mL of water and filtered through double layer of muslin cloth. PDA medium was amended with 10 per cent concentration of plant extracts and autoclaved for 20 minutes (Bhaskaran *et al*., 1988). Mycelial discs of 5 mm diameter from three day old cultures of *P. palmarum* were centrally inoculated on the poured media in 90 mm petri plates. *P. palmarum* was isolated from leaf blight leaves collected from Vadakarapathy panchayath of Palakkad district. Radial growth of the fungus was

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measured after 72 and 96 hours. Mycelial growth in media without plant extract was taken as control. The growth inhibition was calculated based on the growth in the control plate. The growth inhibition was determined by applying the formula of Skidmore, (1976).

$$\frac{C–C_1}{C} \times 100$$

Where, 'C' is the growth of *P. palmarum* in control plate and 'C_1' is the growth of *P. palmarum* in the medium with plant extracts.

The plant extracts which were found to be maximum inhibitory to the mycelial growth of *P. palmarum* were selected to study their effect on sporulation. PDA plate amended with 2 and 5 per cent plant extracts were used for this study. Sporulation was determined by adding 10 mL sterile distilled water to each seven days old culture in the medium amended with plant extract and gently scraping the mycelia with a sterile glass rod to dislodge the spores. The spore suspensions obtained were filtered through sterile cheesecloth into a sterile 50 mL beaker and homogenized by manual shaking. The number of spores was counted using a haemocytometer. The sporulation present inhibition was calculated based on the sporulation in the control plate (Nduagu *et al*., 2008).

Out of the 10 plant extracts studied, extracts of *Lawsonia inermis*, *Cinnamomum zeylanicum, Lantana camara* and *Calotropis gigantea* showed greater inhibition compared to other plant extracts. At 10 per cent concentration, *Lawsonia inermis, Cinnamomum zeylanicum* and *Lantana camara* showed 100 per cent inhibition after 72 and 96 hrs (Table 1). *Calotropis gigantea* leaf extract showed 97.5 and 98.6 per cent inhibition after 72 and 96 hrs respectively. This study showed that when the plant extracts of different concentrations were added to medium, a reduction in the mycelial growth of *P. palmarum* observed, however, the efficacy varied with plant extracts and concentration.

All the selected plant extracts showed reduction in the sporulation of *P. palmarum.* The highest inhibition of sporulation of *P. palmarum* was recorded in the case of *Cinnamomum* and *Lawsonia* (Table 2). *Cinnamomum* exhibited 73.3 and 94.3 per cent inhibition at 2 and 5 per cent concentration respectively. *Lawsonia* showed 75.8 and 97.0 per cent inhibition at 2 and 5 per cent concentration respectively. The results obtained from different studies showed that the crude extracts of *Lawsonia inermis*, *Cinnamomum zeylanicum, Lantana camara* and *Calotropis gigantea* leaves exhibited the highest antifungal activities against *P. palmarum.* The inhibition was expressed as reduced radial growth and sporulation. Bhuvaneswari *et al.* (2010)

Table 1. *In vitro* evaluation of different plant extracts on mycelial growth of *P. palmarum*

Plant extracts	Family	Per cent inhibition after		Mean
		72 hrs	96 hrs	
Allium sativum Linn.	Amaryllidaceae	31.1(26.7)	35.3(33.3)	33.2(30.0)
Azadirachta indica A. Juss.	Meliaceae	29.5(24.2)	35.8(34.2)	32.7(29.2)
Calotropis gigantean R.Br.	Asclepiadaceae	75.0(93.3)	76.4(94.5)	75.7(93.9)
Cinnamomum zeylanicum Blume.	Lauraceae	76.4(94.5)	78.5(96.0)	77.4(95.3)
Lantana camara Linn.	Verbanaceae	75.8(94.0)	78.1(95.8)	77.0(94.9)
Lawsonia inermis Linn.	Lythraceae	78.1(95.8)	79.4(96.6)	78.8(96.2)
Murraya koenigi Spr.	Rutaceae	28.9(23.3)	32.0(32.1)	31.8(27.7)
Nerium odorum Linn.	Apocynaceae	33.5(30.5)	40.8(42.7)	37.2(36.6)
Ocimum sanctum Linn.	Lamiaceae	31.1(26.8)	28.2(22.3)	29.7(24.6)
Piper nigrum Linn.	Piperaceae	36.0(34.5)	37.3(36.8)	36.7(35.7)

C.V. % = 6.3
CD (P= 0.05) for plant extracts = 1.8
CD (P= 0.05) for time intervals = 0.4
CD (P= 0.05) for plant extracts x time intervals = 2.6
Transformation used: $\sin^{-1}$ " p, where 'p' is the inhibition per cent

Table 2. Inhibition of sporulation of *P. palmarum* by plant extracts

Plant extracts	Per cent inhibition of sporulation in concentrations		Mean
	(2%)	(5%)	
Calotropis gigantea R.Br.	54.7(66.6)	67.6(85.5)	60.7(76.0)
Cinnamomum zeylanicum Blume.	58.9(73. 3)	76.2(94.3)	66.3(83.8)
Lantana camara Linn.	54.0(65.5)	68.2(86.3)	66.6(75.9)
Lawsonia inermis Linn.	60.4(75.8)	79.9(96.9)	68.3(86.4)
C.V. % = 5.3			
CD (P= 0.05) for plant extracts = 1.8			
CD (P= 0.05) for concentrations = 0.7			

Transformation used: $\sin^{-1}$ " p, where 'p' is the inhibition per cent

reported the mycelia inhibitory activity by *Cinnamomum zeylanicum* and *Lawsonia inermis* on *P. palmarum* causing leaf blight in palmyarh. Rasmi and Rohini Iyer (2010) reported the antifungal properties of *Andrographis paniculata* and *Lawsonia inermis* against *P. palmivora*, causing bud rot disease in coconut.

Present study shows that the extracts of *Lawsonia inermis, Cinnamomum zeylanicum*, and *Lantana camara,* which showed inhibitory effect on *P. palmarum* can be utilized as new component of Integrated Disease Management (IDM) package for controlling grey leaf blight disease of coconut. Further studies on *in vivo* efficacy of these botanicals and identification of fungitoxic principles needs to be ascertained which will be useful for the formulation of safer and more economical pesticides.

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