

## Yellowing and premature bean dropping in vanilla (Vanilla planifolia Andrews)

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Vanilla (Vanilla planifolia Andrews syn. V. fragrans), a native to the humid tropical rain forests, is now widely cultivated in India for its aromatic vanillin. The initial attractive market of vanilla tempted the farmers to extend the area of cultivation. As the extent of cultivation increased, the crop became susceptible to a number of fungal and viral pathogens which seriously affected the production. Being succulent in nature, it is easily vulnerable to many of the plant pathogenic fungi, some of which causes destructive diseases such as bean rot due to Phytophthora meadii and Sclerotium rolfsii (Suseela Bhai and Joseph Thomas, 2000 and Joseph Thomas and Suseela Bhai, 1999), root rot due to Fusarium oxysporum and Phytophthora meadii and stem rot due to Fusarium sps. (Joseph Thomas et al., 2003). Recently a new disease characterized by yellowing and bean dropping was reported from most of the vanilla plantations in lower elevations of Kerala and Karnataka states. The disease was observed in Calicut, Malappuram, Wyanad, Ernakulam and Idukki districts of Kerala from the month of February to April till the onset of Southwest-Monsoon. A survey conducted to study the incidence and spread of the disease revealed high intensity and wide spread occurrence of the disease. The average crop loss estimated due to this disease ranged from 23-34%.

The disease is characterized by dropping off of dried corolla from the tip of immature beans which otherwise will be attached to the beans till half way through maturity. As the corolla drops off, the exudates from the beans accumulates at the tip, the beans turn yellow and fall off. The bean yellowing is followed by dark brown discoloration of the beans from the tip upwards (Fig-1). Fungal mycelium is found growing at the tip of the beans.

The disease is found associated with high temperature (32.57-35.57°C) and very low relative humidity (45-63.57%) prevailed during the months of February-April (Table1). The yellowing and sudden dropping of beans were severe with the sudden fall in of temperature with the first summer showers. The disease was found negligible in high altitudes where temperature and humidity are maintained under forest cover.

Table 1. Temperature and RH from February - first week of June

Week	Temp	(°C)	RH (%	)	
Year 2003	Max	Min	Max	Min	
Feb 5-11	35.57	26.00	91.57	45.00	V
Feb 12-18	34.5.7	25.57	91.28	47.14	
Feb 19-25	34.28	25.28	91.71	50.14	
Feb 26- March 4	34.71	25.57	90.71	53.35	
March 5-11	34.28	25.42	91.00	49.71	
March 12-18	34.71	25.71	91.28	45.14	
March 19-25	35.00	25.71	90.28	45.57	
March 26-April 1	35.00	25.85	91.14	49.71	
April 2-8	33.85	26.42	91.57	55.85	
April 9-15	34.00	27.00	93.00	55.50	
April 16-22	34.14	26.42	91.71	56.85	
April 23-29	34.42	26.85	91.71	56.64	
April 30-May 6	33.71	26.71	91.71	55.85	
May 7-13	33.71	26.85	91.85	54.42	
May 14-20	32.57	26.71	91.85	63.57	Awh.
May 21-27	34.57	27.00	92.00	55.52	
May 28-June 7	34.28	26.71	91.71	54.78	

A disease survey was conducted in vanilla plantations of Calicut and Wyanad districts of Kerala during the months of March to April 2003. Twenty-two plantations including 16 in Calicut and 6 in Wyanad were surveyed for the intensity and spread of the disease. Besides, five plantations in Muvattupuzha area of Ernakulam district and eight plantations in Malappuram district. Crop loss assessment from the infected areas showed that the per

centage loss due to this disease ranged from 23.25-34 and in severe cases it extended up to 57

A laboratory study was conducted for the isolation of the pathogen. The disease specimens after thorough surface sterilization were plated in PDA, PVPH and Nutrient Agar media. A fungal growth was obtained in PDA, whereas no growth was obtained either in nutrient agar or in PVPH medium. The same fungus was isolated repeatedly from all the samples tested. Similarly the yellowing and browning affected bean tissues, in the initial stages of infection, on direct microscopic examination also showed the presence of fungal mycelium and conidia of *Colletotrichum* sp. Irrespective of the place of collection all the infected beans showed similar fungal conidia which was found germinating inside the tissues.



Fig.1. Symptoms of premature yellowing and bean dropping

The fungus on reinoculation to healthy immature beans at a temp. of  $25 \pm 1^{\circ}$ C and also at laboratory temp. of  $28\text{--}30^{\circ}$ C under *in vitro* conditions reproduced the same symptoms as under natural conditions in 7 days. The inoculated beans showed yellowing followed by browning at both ends as occurred in nature. It was also found to be pathogenic to leaf, although under field conditions leaf infection could be rarely observed during the particular period .

In PDA the colonies were at first white in colour, which later changed into gray white with black streaks on the lower surface of the plate. The conidia were almost cylindrical and aseptate. Hyphae were hyaline and septate, measured 2.6-3.9  $\mu$ m in width and conidia 13-

20.8 x 2.6-3.9  $\mu$ m. The temperature and pH studies showed that the fungus could survive at pH ranging from 4-9 (5.99 mm at pH 4 and 7.50 mm at pH 8) and at temperature between 25-35°C (Table 2 and 3). The fungus was identified as *Colletotrichum vanillae* Massae.

Table 2. Effect of temperature on growth of the fungus

Temperature (° C)	15	25	30	35
Growth rate (mm)	0.55	7.6	5.72	2.96

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pH	4	5	6	7	8	9
Growth rate (mm)	5.99	7.36	7.16	7.22	7.50	6.68

The fungus was tested for its sensitivity to fungicides and antagonistic rhizobacteria under *in vitro* conditions (Table 4 and 5). Seven different fungicides viz. Bordeaux mixture, Copper oxychloride, Metalaxyl- mancozeb, Thiophanate methyl, Carbendazim, Mancozeb, and a combination of Carbendazim- mancozeb and twenty rhizobacterial isolates were tested under *in vitro* conditions to evaluate their efficacy in inhibiting *Colletotrichum vanillae* to control the spread of the fungus.

Table 5. Effect of rhizobacterial isolates on Colletotrichum vanillae

	Rhizobacterial isolates	Colletotrichum  vanillae  Inhibition(%)					
	isolates						
	IISR 6	63.7					
	IISR 13	31.5					
	IISR 51	69.8					
	IISR 853	49.6					
	IISR 859	39.5					
	IISR 906	39.5					
	IISR 907	64.5					
	IISR 909	73.8					
	IISR 910	51.6					
	IISR 912	65.7					
	IISR 913	59.7					
	IISR 914	39.5					
	IISR 915	63.7					
	IISR 147	49.6					
	IISR 148	54.4	*				
	IISR 149	39.7					
	IISR 150	46.4					
	IISR 151	49.6					
	IISR 152	87.9					
	IISR 153	36.3					

The results of fungicide sensitivity studies indicated that thiophanate methyl (100ppm) and carbendazim were highly inhibitory to the fungus even at 250 ppm

Table 4. In vitro effect of fungicides on Colletotrichum vanillae

Fungicides			% inhibit	% inhibition				
								ED 90
Concentration (ppm)	50	100	250	500	1000	1500	2000	Value
Bordeaux mixture	10.78	17.5	28.28	50.78	100	100	100	900
Carbendazim	92.63	94.86	100	100	100	100	100	47.96
Carbendazim+ Mancozeb	81.79	86.9	88.0	89.7	91.84	95.39	100	493.64
Copper oxychloride	0.00	8.94	51.30	93.0	100	100	100	450
Metalaxyl-mancozeb	21.7	52.36	77.8	82.89	86.97	91.45	100	1004.46
Mancozeb	7.89	11.18	13.42	15.78	23.94	55.0	59.2	2368
Thiophanate Methyl	96.84	98.8	100	100	100	100	100	46.46

The ED <sup>90</sup> value was found to be below 50 ppm for these fungicides. While other fungicides are effective only at higher concentrations. Among the twenty rhizobacterial isolates tested, IISR 152 and 909 were inhibitory to *Colletotrichum vanillae* showing an inhibition rate of 87.9 and 73.8%, respectively. Isolates IISR 6, IISR 51, IISR 907, IISR 912 and IISR 915 are also found promising giving an inhibition up to 62-69%.

Yellowing and fruit shedding due to Fusarium species was reported by Joseph Thomas et al. (2003). This disease appears on beans of 5-7 months old during August to October months. The symptom appear in the form of yellow colour at the tip of the beans which slowly extend towards the pedicel and leads to splitting of the beans at the tip followed by brown colouration of the affected portions. But in the present study none of the samples showed the presence of Fusarium sp. either in the tissues under microscopic examination or in isolations using different culture media. Similarly no splitting of the bean was noticed in any of the beans affected by yellowing which occurred during Feb-May months while pollination is in progress. Hence the

present work confirmed the involvement of Colletotrichum vanillae in causing yellowing and premature bean dropping of vanilla during stress conditions of high temperature and low humidity. As the disease is found to be associated with airborne fungal infection, spraying fungicides which are found effective under in vitro conditions can be used to manage the disease to a greater extent.

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Indian Institute of Spices Research, Marikunnu P. O Calicut, 673 012 Kerala, India

Suseela Bhai R., Ishwara Bhat, A., Anandaraj, M.