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Occurrence of fungal diseases in vanilla (*Vanilla planifolia andrews*) in Kerala

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Abstract

A study was carried out to evaluate the distribution and incidence of various fungal diseases on vanilla (*Vanilla planifolia* Andrews) in major vanilla growing tracts of Kerala. The study included both survey and assessment of diseases as and when reported by farmers. Survey was conducted during April, May and August 2003, March 2004 and November 2005. A total of 111 locations (72 locations during survey and 39 locations from farmer's reports) distributed in six districts were subjected for the study. The major disease noticed in survey during April -May 2003 was yellowing and premature bean shedding (YPBS). The disease was found associated with *Colletotrichum vanillae* Massae, predisposed by high temperature and low relative humidity. During August 2003, *Phytophthora* incited stem and bean infection was noticed in isolated areas in Kozhikode and Idukki districts. Survey during March 2004 at Kozhikode revealed the recurrence of flower shedding and YPBS, besides root rot caused by *Fusarium oxysporum* f. sp. vanillae . Survey during November 2005 in Wyanad area revealed the prevalence of stem and root rot incited by *Fusarium* species along with leaf axil rot incited by *Colletotrichum* sp. In addition, *Rhizoctonia solani*, *Cylindrocladium quinqueseptatum* and *Mucor racemosus* were also isolated as pathogens from different symptomatic parts. Other minor infections noticed were leaf rot, bean rot and brown rot. *Fusarium* and *Colletotrichum* sp. were the predominant pathogens found to be associated with most of the infections on vanilla. The association of *Mucor* sp. with leaf and bean rot and *C. quinqueseptatum* with brown rot were observed as new reports of its kind on vanilla.

Keywords: Brown spot, Characterisation, *Colletotrichum vanillae*, *Mucor recemosus*, pathogenicity, *Phytophthora meadii*, root rot, stem rot, *Vanilla planifolia*.

Introduction

In Kerala vanilla is grown from sea level to an altitude of about 4500 feet above MSL. It is grown as an intercrop along with arecanut, coconut, coffee and black pepper, in homestead gardens and as a pure crop in standards such as Glyricidia, Plumeria, Dadaps and concrete or stone poles. During initial years of cultivation diseases were not common. But as the extent of area under cultivation is increased, the crop become susceptible to a number of fungal pathogens, some of which resulted in total destruction of vines. All parts of the plant are susceptible to attack by the pathogens. Generally, fungal diseases are wide spread in closely planted gardens having thick shade, intensive management, frequent irrigation and in plantations where no phytosanitory measures are adopted. Fungal infections often lead to rotting of plant parts

or wilting of the entire vine as in the case of stem or root rot diseases. Species of *Phytophthora*, *Fusarium*, *Sclerotium*, *Calospora* and *Colletotrichum* are the commonly occurring pathogens reported to cause serious damages in vanilla (Purseglove *et al* 1988). In the present study an effort was made to investigate the status of various fungal infections on vanilla in different vanilla growing tracts of Kerala.

Materials and methods

Survey

The survey was conducted in vanilla gardens of Kozhikode, Ernakulam, Idukki and Wyanad districts from 2003-2005. During the survey samples of disease affected plant parts including stem, leaf, fruits and roots were collected. In addition to survey, the diseases as and when reported by farmers from various locations during different months were also studied.

Isolation, pathogenicity and identification of pathogens

The samples collected during the survey as well as those brought by farmers were subjected to pure culture isolation of the pathogen(s). The fungal cultures thus obtained were tested for pathogenicity and identified based on their cultural and morphological characteristics. For this purpose, mycelial disc of 3mm size were cut with sterile cork borer from the periphery of 72h old actively growing culture and inoculated centrally on the PDA plates and incubated at 24-25ºC. After 72-120 h, they were examined for colony morphology as well as microscopic characteristics for identification of organisms.

Rooted cuttings of vanilla maintained in the green house were used for pathogenicity tests. They were washed thoroughly in running water to remove dust particles, surface sterilised with 0.1% Mercuric chloride solution and then washed with several changes of sterile distilled water. The cuttings were then inoculated with actively growing culture discs of representative test isolates. The fungal culture discs were kept moistened by keeping a wet cotton wad over it and incubated at room temperature. Simultaneously, control was also maintained with plain agar disc and incubated as above. Pathogenicity was also cross tested on other reported hosts in case of new pathogen.

Results and discussion

Survey

The survey was initiated during April 2003 followed by subsequent surveys in May 2003, August 2003, March 2004 and November 2005. The age of the surveyed vanilla vines ranged from 2-5 years. Both organic and inorganic system of cultivation was followed by farmers. During March- May 2003, high intensity of yellowing and premature bean shedding was observed as the major problem on bearing vines in all the areas irrespective of the management practices adopted by the farmer (Fig. 1) . Twenty-two plantations including 16 in Kozhikode and six in Wyanad were surveyed, 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 percent crop loss due to this disease ranged from 23.25-34 % and in severe cases it extended up to 57%. Incidence of the same was also reported by farmers even from February till the onset of south west

Fig. 1. Yellowing and premature bean shedding (YPBS) caused by *Colletotrichum vanillae*.

monsoon showers in May . Of the 16 reports from farmers from various locations, 11 are of yellowing and premature fruit dropping which is the initial stage of YPBS infection. But the disease has been subsided with the onset of monsoon.

The third survey was conducted during August 2003. The survey was mainly concentrated on Kozhikode and Idukki districts. 20 locations were surveyed. This includes eight locations in Kozhikode and 12 locations in Idukki of which 15 locations were found free from fungal infection. In remaining locations, the main disease noticed was stem and bean rot caused by *P. meadii*. One plantation at Myladumpara in Idukki district showed a very high intensity of *Phytophthora* infection and one plantation at Muthappannpuzha in Kozhikode district showed root rot caused by *Fusarium oxysporum* f sp. *Vanillae* (Fig. 2).

Fig.2. Stem rot and root rot of vanilla caused by *Fusarium oxysporum* f sp. *Vanillae*

The fourth survey was conducted during March 2004 in Kozhikode district. Eight locations were covered. Flower dropping and premature bean shedding were the major recurrent problems noticed. Root rot was also noticed in two plantations at Chempukadavu area of Kozhikode district. The final survey was conducted during November 2005 at Wyanad district of Kerala where root rot was reported as a threatening problem. During the survey 16 plantations were surveyed. All the plantations in Wyanad lie in an altitude of 750-790 M above MSL. Root rot, wilt and stem rot were found to be the major symptoms in most of the plantations. Incidence ranged from 5-100%. Two of the plantations were totally wiped out due to root rot (Fig. 3). The symptoms were yellowing and drying of the stem and leaves. Only standards were remained there as a sign of vanilla planted. Of the 16 plantations surveyed five plantations showed no fungal infection and were found absolutely healthy.

Fig.3. A view of plantation wiped out due to root rot

In Wyanad more than 65% of the farmers have adopted organic system of cultivation. The most commonly used organics were neem cake, pongamia cake, bone meal, Panchagavya, and bio fertilizers. The age of the crop ranged from 2-5 years and intercropped with arecanut, black pepper and coffee. Glyricidia was used as the main standard. Most of the farmers followed intensive management where as a few maintained the plantation with minimum tillage practices.

Isolation, pathogenicity and identification of pathogens

Representative samples including root, stem and leaves were collected from each plantation and subjected to pathogen isolation. Fungal isolates obtained from each diseased sample

were sub-cultured into PDA slants and maintained in the repository after giving the IISR number prefixed by V. The details of the isolations made from Wyanad area is given Table 1.

Colletotrichum vanillae was found associated with all the infected beans affected by premature yellowing and bean shedding samples. Occasionally *Phytophthroa* species was isolated from root, stem and bean rot

Table-1 details of isolations made from Wyanad

Sl.No.	Symptoms	Fungi isolated	IISR repository No.
1	Stem rotting drying	*Fusarium* sp.,	IIISR V125
		C. gloeosporioides	IIISR V126
2	Leaf rot	*C. vanillae*	IIISR V127
3	Yellowing and drying of stem.	*C. gloeosporioides*	IIISR V128
4	Leaf rot	*F. oxysporum* f.sp *vanillae*	IIISR V154
		Fusarium sp.	IIISR V155
5	Leaf rotting	*Fusarium* sp.	IIISR V156
		-	
6	Leaf Yellowing	*F. oxysporum* f.sp *vanillae*	IIISR V158
		C. gloeosporioides	IIISR V159
		Fusarium sp.	IIISR V160
7	Stem rot & leaf drying	*C. vanillae*	IIISR V130
		C. gloeosporioides	IIISR V131
8	Leaf drying	*Fusarium* sp.	IIISR V146
		Rhizoctonia	IIISR V147
9	Stem rot	*C. vanillae*	IIISR V132
		Mucor racemosus	IIISR V133
10	Stem rot	*F. oxysporum* f.sp *vanillae*	IIISR V135
11	Leaf rotting	*F. oxysporum* f.sp *vanillae*	IIISR V161
12	Yellowing of stem	*Mucor racemosus*	IIISR V136
		C. gloeosporioides	IIISR V137
13		*Colletotrichum* sp.	IIISR V148
14	Bean rotting	*Fusarium* sp.	IIISR V176
		Mucor racemosus	IIISR V177
15	Stem rot	*C. gloeosporioides*	IIISR V138
16	Leaf yellowing and rotting	*Fusarium* sp.	IIISR V163
		C. gloeosporioides	IIISR V164
17	Aerial root and stem drying	*Fusarium* sp.	IIISR V186
	Stem Yellowing	*Rhizoctonia* sp.	IIISR V187
		Trichoderma sp.	IIISR V188
		C. gloeosporioides	IIISR V139
		Fusarium sp.	IIISR V140

Sl.No.	Symptoms	Fungi isolated	IISR repository No.
18	Stem rotting	*C. gloeosporioides*	IIISR V141
		C. gloeosporioides	IIISR V142
		Fusarium sp.	IIISR V143
19	Lichens on surface	*C. vanillae*	IIISR V165
20	Stem rot (node)	*C. vanillae*	IIISR V166
21	Leaf rotting	*Fusarium* sp.	IIISR V149
		Fusarium sp.	IIISR V150
		Fusarium sp.	IIISR V151
22	Bean rotting	*Fusarium* sp.	IIISR V178
		Fusarium sp.	IIISR V179
23	Stem rotting	*Fusarium* sp	IIISR V152
		Fusarium sp	IIISR V153
24	Leaf rotting	*T. harzianum*	IIISR V167
		Trichoderma sp	IIISR V168
25	Leaf rotting	*Fusarium* sp	IIISR V169
		C. gloeosporioides	IIISR V170
26	Leaf rotting	*Fusarium* sp.	IIISR V171
		C. gloeosporioides	IIISR V172
		C. gloeosporioides	IIISR V173
27	Stem Yellowing	Unidentified-no sporulation	IIISR V144
28	Stem rotting	*C. gloeosporioides*	IIISR V145
29	Leaf drying	*Mucor racemosus*	IIISR V180
		Unidentified-no sporulation	IIISR V181
30	Leaf yellowing	Unidentified-no sporulation	IIISR V174
		C. gloeosporioides	IIISR V175
31	Aerial root drying	*Trichoderma harzianum*	IIISR V184
		F. oxysporum f.sp *vanillae*	IIISR V185
32	Bean tip rot	Unidentified-no sporulation	IIISR V182
		Unidentified-no sporulation	IIISR V183

diseases and identified as *P. meadii* (Fig. 4). Among the 64 isolates obtained from Wyanad area, 24 isolates were of *Fusarium* species and 20 isolates were of *Colletotrichum* sp. The rest of the isolates belonged to *Rhizoctonia, Mucor* and *Trichoderma* and some non sporulating fungi which are non-pathogenic. Table 2 shows the organisms associated with different disease symptoms.

During September 2004, a new disease of vanilla beans was noticed in Chempukadavu area of Kozhikode District (Kerala) where vanilla is grown in a large scale intercropped with coconut (*Cocos nucifera* L.), arecanut (*Areca catechu* L.) and clove (*Syzygium aromaticum*) . The intensity of infection ranged from 10%- 90% on the beans. The disease was identified as brown rot caused by *Cylindrocladium quinqueseptatum* (Fig.5) (Suseela Bhai & Anandaraj 2006)

All the fungal isolates obtained were tested for pathogenicity by inoculating on to excised vanilla cuttings. Infection was observed within 7 days. The *Colletotrichum* sp. showed pathogenicity was identified as *C. vanillae* Massae because of their host specificity. Other

Table 2. Vanilla disease symptoms & associated organisms

Disease Symptoms	Associated Organism (s)	Period of occurrence
Aerial root drying	*Fusarium oxysporum* f sp. *vanillae, Rhizoctonia solani*	
Root rot	*Fusarium oxysporum* f sp. *vanillae*	August - April
Stem rot & drying	*Fusarium oxysporum* f sp. *vanillae*	
Stem tip rot	*Fusarium* sp.	
Stem rot & yellowing	*Fusarium* sp, *Rhizoctonia* sp.	
Yellowing & fruit rot	*Fusarium oxysporum* f sp. *vanillae*	
Yellowing	*Fusarium oxysporum* f sp. *vanillae*	
Flower drop	*Fusarium oxysporum* f sp. *vanillae*	
Leaf spots	*Colletotrichum vanillae*	February --May
Leaf axil rot	*Colletotrichum vanillae*	
Leaf rot	*Colletotrichum vanillae*	
Flower shedding	*Colletotrichum vanillae*	
Premature Yellowing & bean shedding	*Colletotrichum vanillae*	
Bean shedding	*Colletotrichum vanillae*	
Stem rot	*Colletotrichum vanillae*	
Bean rot	*Phytophthora meadii*	June- September
Root rot	*Phytophthora meadii*	
Stem rot	*Phytophthora meadii*	
Brown spot ,	*Cylindrocladium quinquiseptatum*	September-December
Leaf rot, Bean rot	*Mucor racemosus*	

Fig.4. Bean rot caused by *Phytophthora meadii*

Fig. 5. Brown rot of vanilla caused by *Cylindrocladium quinqueseptatum*

isolates of *Colletotrichum* belonged to *C. gloeosporioides.*

The *Mucor* sp. was also isolated from rot affected leaf, stem and beans from three plantations and proved pathogenicity as per Koch's postulates (Fig. 6). The species was identified as *M. racemosus* which is the first report of its kind on vanilla. Mycelium of the fungus is yellowish, very extensive and

Fig. 6. Leaf rot caused by *Mucor racemosus*

septate in older aerial hyphae. Budding cells are formed in media (Domsch & Gams, 1970). Mycelium breaks up into oidia is characteristic of *Mucor recemosus.* It was earlier isolated from rotted apples and oranges. The species isolated from vanilla was found pathogenic on apple also.

Stem rot characterised by the rotting of the internodal area, which initiated on the nodal regions and extended to both ways. This was the severe disease problem noticed in many of the vanilla gardens (Fig 7). Both *Fusarium* and *Colletotrichum* sp. were isolated from these infected parts. Root rot is the major devastating problem characterised by the drying and rotting of the roots touching the soil. Even aerial roots also showed drying. *Fusarium* was isolated from these roots in addition to its isolation from stem and leaves. The *Fusarium* species isolated from root and stem rot infection could be identified as *F.oxysporum* f.sp *vanillae.*

Fig. 7. Leaf axil rot caused by *Colletotrichum* sp.

Earlier studies by Tombe *et al.* (1992) reported stem rot caused by *Fusarium oxysporum* f.sp. *vanillae.* There were also reports of *Fusarium oxysporum* Schlecht f.sp. *vanillae* causing serious root rot of *Vanilla planifolia* (Childers & Cibes 1948). This disease caused severe crop losses in Java, Bali, North Sumatra and North Sulawesi and Indonesia and the occurrence of the disease was noticed throughout the year. In the present study also, among the pathogens isolated, *Fusarium* is found to be the more predominant organism in all most all vanilla gardens.

Root rot was also found to be a serious problem in many of the vanilla growing areas (Deqvaire 1976). *Fusarium* rot or wilting caused by *Fusarium oxysporum* was reported as the most serious of diseases of vanilla (Phillip 1980). The disease is more prevalent in younger plantations especially during the monsoon season. The fungus also occurs in root, shoot tip, pods and leaves of the plant. Infection at collar and roots cause foot rot and wilting of vine and vascular browning of stems in wilted plants. Affected plants get detached from the soil. Excess of moisture or shade, prolonged rainy weather, insufficient drainage and overcrowding all favour the spread of the disease. Great damage was caused in Seychelles in the 1890s, when the vines were planted too close together (Alabouvette *et al.* 1996).

Rhizoctonia solani was also isolated from aerial roots individually or along with *Fusarium* sp. The pathogenicity tests individually and along with *Fusarium* sp. showed yellowing and rotting of the stem and roots of which there was an aggravation of the disease in combined inoculations. This is supported by the studies conducted by Alconero & Santiago (1969). They showed that *Rhizoctonia solani* predisposed the roots of *V. planifolia* and *V. phaeantha* to infection by Fov. and *Rhizoctonia. Rhizoctonia* being an aggressive pathogen, usually invaded tissues ahead of *Fusarium*. This is also in accordance with the present isolation of *Rhizoctonia* from certain samples.

Phytophthora species was isolated from rot affected stem, beans and a root during post monsoon season is in accordance with the earlier reports. Tsao & Mu (1987) reported the incidence of root rot of vanilla along with leaf blight in French Polynesia where *Phytophthora* sp. was identified as the causal organism. *P. meadii* caused rotting of beans, leaves and stems. The disease is more severe during the monsoon especially in shaded plantations and in poorly drained soils (Suseela Bhai & Joseph Thomas 2000).

Yet another malady characterised by premature yellowing and bean shedding was found to be associated with *C. vanillae* (Suseela Bhai et al. 2006). This is entirely different from the bean yellowing incited by *Fusarium* as reported by Joseph Thomas *et al.* (2003). The occurrence of this infection was found during post monsoon season.

Colletotrichum species associated with leaf axil rotting was found to be a new infection. This may be due to the wide use of Glyricidia as a standard for vanilla which is found prone to infection by *C. gloeosporioides*. This was reported recently by Lenne & Sumberg (1986). Thus the present study on the status of various fungal infections on vanilla is supported by earlier reports from Puerto Rico and Madagascar and other vanilla growing countries. However the brown rot caused by *C. quinquiseptatum* , leaf, stem and bean rot caused by *M. racemosus* as well as premature yellowing and bean shedding caused by *C. vanillae* are hither to un-reported from any of the vanilla growing countries and are found to be new reports of its kind on vanilla.

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