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Study of disease spectra, pathogen characterization and their management of cane seedlings in nursery in Central Western Ghats of Karnataka

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Abstract
Canes (*Calamus spp*.,) are high export valued evergreen non timber forest producing species in India fetching high revenue. Its resources are fast depleting in recent past due to disease induced seedling mortality, heavy premature defoliation, growth and vigour impairment keeping over exploitation.The investigation to assess disease spectra, growth lossand diseases management in Canes was done during 2017 to 2018 in 17 rattan nurseries covering 5 districts under 3 bioclimatic zones of Western Ghats part of Karnataka. Disease spectral study revealed fungal diseases *viz*., leaf spots, leaf blights, sooty mold and root rot disease. Fungal pathogenic spectra included *Pestalotiopsis oxyanthi*, *Alternaria dianthi*, *Capnodium salicinum* and *Fusarium solani.* Of four diseases, leaf blight was observed to be more damaging to seedling.Among all 17 forest nurseries, Bellanggi nursery had maximum severity (34.05%) followed by Megaravalli (32.48%) and Kallage (29.91%) nurseries and least severity in Sampaje nursery (11.02%). In field evaluations, among the 6 fungitoxicants Propiconazole or Carbendazim @ 0.2 per cent gave effectively control all foliar diseases of rattan.

Keywords: *Calamus spp.*, PDI, leaf blight, growth loss, propiconazole and carbendazim

Introduction
Rattan (*Calamus spp.* Linnaeus) is a high export valued evergreen non timber forest produce belonging to the subfamily Calamoideae of family Arecaceae and is characterized by fruit bearing scales [1]. The climbing palm of canes grows up to 10-15 m height and 6-120 mm stem diameter under different ecological conditions. They occur at different altitudinal and rainfall zones and are distributed from 2000 m above MSL. Species are found in wet evergreen forests with an annual rainfall of 5000 mm, while others are found in areas with an annual rainfall of 750 mm only. India has a good representation of canes with 5 genera (Calamus, Daemonorops, Korthalisa, Plectocomia and Zalacca) and 70 species. Which are distributed along the wet evergreen forests of the Western Ghats of Peninsular India, North-Eastern Himalaya and Andaman and Nicobar Islands. In Indian subcontinent, it found in the natural forest areas of Assam, Arunachal Pradesh, Karnataka and Kerala states and is widely distributed in evergreen, semi-evergreen and moist deciduous forests. Among these, only one genus, *Calamus* in Peninsular India [2] and Karnataka has a rich accumulation of 15 species. Madikeri district of Karnataka is enriched with 12 species.

Rattan is of great economic importance in handicraft and furniture making because of its richness in fibre, with suitable toughness and easy for processing. They are highly valued and have social and economic importance because of their unique characteristics such as strength, durability, looks and bending ability; they are regarded as “green gold”. Current trade is multimillion dollar business. Indian cane furniture industries produce materials worth Rs. 50 million with Rs. 5 million export value Direct employment to more than half a million in SE Asia.

Urban people are employed in the small-scale industries and cottage industries manufacturing cane furniture and other articles. Inspite of high economic and medicinal value, the canes exist mostly in wild that too in less accessible natural forest in Karnataka. Because of high demand for rattan products worldwide and its collection from wild habitat, rapid deforestation and threat to the survivability of most of the species of canes [2]. Of the approximately 600 species of rattan, 117 are recorded as being threatened to some degree; of these, 21 are endangered, 38 are regarded as vulnerable, 28 as being rare and 30 as indeterminate (IUCN). North-Eastern states alone accounts for 4 genera and more than 20 species. Out of these 20 species, 14 species are being threatened including eleven endemic species.

Indiscriminate extraction of canes, climatic vagaries, biotic and abiotic stress (erratic rainfall, fire, grazing, unscientific harvesting, pests and diseases) is affecting its productive potential. Of them, vulnerability of canes to diseases in nurseries, plantations and natural stands is evident. Initial survey observations show diversified diseases, disease induced mortality and growth impairments which found to result partial to complete failure of several nurseries. The status of 8 cane species under threat category in Karnataka is the best indicator.

Fungal pathogens are considered as one of the most serious pathogens causing a significant reduction in canes palm growth, development and production. In Western Ghats, canes developing forest deportment nurseries encounters several constraints among which the wide spread of fungal diseases presenting a serious threat for growth and development of rattan species. Several important fungal pathogens have been isolated and identified as a causal agent of damaging diseases, including leaf blight disease (*Collectotricum sp*., *Pestalotiopsis sp*., and *Phomopsis sp*.), leaf spot (*Pestalotia*) and root rot (*Fusarium oxysporum* and *Fusarium solanii*) [3&4]. Most of these diseases have been concentrated in the rattan seedling production in without shade or open condition nurseries, where the high level of temperature and humidity could contribute to the spread of these fungal infections. But forest department personnel are facing severe problem in timely controlling seedling diseases in nurseries. Hence, the present investigation is proposed with the following objective are documentation of disease spectra, pathogen characterization and their management of cane seedlings in nursery.

Materials and Methods

The investigation survey work was done in 17 different rattns growing nurseries of differnt ranges under 3 ecoclimatic zones having 5 districts as depicted. To assess the diseas and growth loss assessment was conducted durning the period from April 2017 to March 2018.

Survey of disease incidence

Each site was inspected during April 2017 to March 2018. Percentage of infection and symptoms with leaf blight, leaf spot, root rot and sooty mold was recorded. The Disease Incidence (DI), Per cent Disease Index (PDI) was calculated according to the following formula:

$$DI\ (\%) = \frac{\text{No. of seedlings affected}}{\text{Total no. of seedlings observed}} \times 100$$

$$PDI\ (\%) = \frac{\text{Sum of numerical ratings of all the leaves}}{\text{Total number of leaves observed} \times \text{Maximum grade used}} \times 100$$

Identification of fungal isolates

The identification of fungal isolates was performed on Potato Dextrose Agar (PDA) medium plates by scientist [5]. Single fungal colonies were picked from hyphal tips and grown on PDA for 7 days at 25 ^{0}C for further examination of the mycological characteristics.

Morphological characterization

Colonies were described according to morphological characteristics, examined under microscope and the frequency (%) of the isolated fungi was recorded.

Pathogenicity test

A pathogenicity test was conducted for each fungal isolate separately. Canes healthy seedlings were inoculated by the pathogen under greenhouse conditions (25 to 29 ^{0}C and 12/12-h light/dark). From inoculated areas the fungus was re-isolated and colonies were maintained in PDA for morphological characterization.

Disease management

The seedlings were fortnightly treated with 6 fungitoxicants at different concentration and an evaluation of these chemicals was done at Bellangi nursery, Kumata. By seeing the effective fungicides, plant extracts and bio agent as evidenced from *in vitro* studies, highly effective two were used for field evaluation under nursery conditions. In this field experiment use 2 fungicides of Tilt and Bavistin @ 0.2 per cent, 2 botanicals of *Azardirachta indica* and *Annona squamosa* @ 10 per cent and 2 bio agents like, *Trichoderma harzianum* and *Bacillus subtilis*. For bio control agent a suspension of $1x10^6$ Cfu/ml was used as spray. The test seedlings were given 2 sprays at 30 days interval. The observations on disease severity was recorded once before first spray and twice, one each after 30 days of first and second spray. Per cent Disease Index was estimated based on severity scale. Per cent reduction in the treatments over control was also assessed.

Results and Discussion

The results revealed that among the different nurseries and irrespective of disease showed high mean percent combined foliar disease incidence and per cent disease incidence was recorded in Katgal nursery (31.90 & 32.95%) fallowed by Megaravalli nursery (29.75 & 31.14%) and Sringeri (26.68 & 28.33%) and least disease incidence and per cent disease incidence was showed in Sampaje nursery (9.76 & 11.90%) (Table 1). Probably poor management and chance of high relative humidity and shortened and overlapped pathogenic life cycle favoured by prolonged leaf wetness period by drizzling rain, high relative humidity, built up due to close stacking of seedling, sprinkler irrigation, poor management and river might have created conducive microclimate for multiple host disease prevalence. [6], record of *Cercospora* leaf spot and *Meliola* sooty mold on oil palm with high incidence (11.26% and 51.30%.) and likewise, disease survey by different researchers reported leaf spot recorded highest disease incidence and severity (80.36% and 40.67%) on Coconut [3].

Symptoms of diseases

The symptoms of different diseases developed on seedlings of rattan species have been documented. Most of the diseases were noticed in young, medium and matured leaves. leaf blight of canes leaves were small circular to irregular purplish to brownish necrotic areas with yellow halo enlarged gray areas, leaf spot symptoms were circular white spots with brown margin, root rot symptoms were brown to buckish discolouration near the soil line root decay, yellowing and death and sooty mold symptoms were black sooty type growth on upper surface of leaves and further covered entire leaf lamina. The symptoms are in accordance with earlier reports on symptoms of *Pestalotiopsis* leaf spot, *Phomopsis* leaf spot, *Sphaerodothis* leaf blight and *Pellicularia* leaf blight symptoms showed greyish brown, water-soaked lesions, which later spread and coalesced to form oval to circular necrotic lesions with dark greyish brown irregular margin, small spindle shaped, pale yellow lesions on the

leaves, dark brown with a yellow halo [6]. Usually, two to three such large necrotic patches were observed in one leaf of on *C. drensefieldii*, *C. pseudotenuis*, *C. thwaitesii*, *C. vattayila* and *C. rotang* was accounted to overcrowding of seedlings, high temperature, use heavy chemical fertilizer, seeds and seedlings collected from disease pruned area, seedling grown in open condition and non-practice of fungicidal treatments [4].

Organism Identification

Leaf spot was caused by pathogen on different hosts *viz*., *Pestolotiopsis oxyanthi* for leaf spot of *C. nagbettai*, *C. prasinus and C. thwaitesii*; Likewise for leaf blight, *Alternaria dianthi* for blight of *C. nagbettai*, *C. prasinus*, *C. vattayila*, *C. dransfieldii* and *C. thwaitesii*. Pathogenic fungi of root rot, *Fusarium solani* for root rot of *C. nagbetaii, C. prasinus and C. thwaitesii*. Likewise, *Capnodium salicinum* for sooty mold of *C. nagbettai, C. prasinus and C. thwaitesii*. Present findings get supportive endorsement by the findings leaf blight of *C. nagbettai* and *C. thwaitesii* caused by *Coniothyrium dalbergia* and *Chaetomium pachypodioides* were reported [7]; leaf blight and leaf spot on *C. thwaitesii* caused by *Colletotrichum gloeosporioides*, *C. crassipes*, *Guignardia calami* and *Fusarium pallidoroseum* [4].

Management of disease

In vivo fungitoxicant evaluations against *Alternaria* causing leaf blight and *Pestalotiops is* causing leaf spot was carried out by the fungitoxicants which proved as the best in *In vitro* assays (Propiconazole 25% EC, Carbendazim 50% WP, *Trichoderma harzianum*, *Bacillus subtilis* and fresh seed extract of *Azardirachta indica* and *Annona squamosa*). Evaluations by two foliar sprays at fortnight intervals in Bellangi nursery revealed the effectiveness of 0.2% Propiconazole (Tilt) could suppress leaf blight of *Calamus spp.,* (32.06 to 41.40%) over control followed by 0.2% (Bavistin) Carbendazim (28.61 to 34.44%). Unlike this, the longevity of anti-pathogenic efficacy retention was not more than 30 days in neither plant extracts nor bio-agents application. The best performance of Propiconazole 25% EC can be attributed to high fungicidal property with long lasting anti-pathogenic activity on the host.These findings are in concurrence with reports on effectiveness of carboxin, Mancozeb, Maneb, Bavistin, Bropineb, Zineb, Chlorothalonil, Captofol, Captan, Benomyl and carbendazim in reducing severity of leaf spot, leaf blight and shot holes of *Calamus sp*., *Calamus thwaitesii*, *C. trachycoleus*, *C. manan* and *C. caesius* are reported several researchers [8&9]. The result are line in these 0.02 per cent of Bayleton or Topsin-M managed leaf blight, leaf ring spots and leaf white spot diseases of *Calamu stetradactylus* [10].

Table 1: Mean Disease Incidence and Per cent disease incidence (PDI) in different nursery of different cane species

District	Nursery names	Mean DI (%)	Mean PDI (%)
Madakere	Sampaje	9.76	11.90
	Karike	13.46	15.70
	Kaveri	15.68	18.70
Shivamogga	Ennehole	21.40	20.00
	Megaravalli	29.75	31.14
	Chandhikamba	20.48	15.87
Chikka mangaluru	Thunga	24.38	28.12
	Thunga	18.61	16.16
	Kerekatte	21.97	24.51
Dakshina kannada	Kallage	25.68	28.33
	Mundajekapu	20.18	20.78
	Ennekallu	18.80	18.28
Uttara kannada	Bellangi	31.90	32.95
	Kengre	18.18	17.93
	Bhatkal	21.84	23.36
	Hebri	21.58	21.88
	Saravathi	24.15	24.76

Table 2: Evaluation of fungitoxicants against foliar diseases of Cane *spp* in Bellangi nursery

Treatment		Concentration	Per cent disease Index (PDI)			Per cent reduction in PDI over control	
			Before spray	30 days after I spray	30 days after II spray	30 days after I spray	30 days after II spray
T_1	Control	-	21.00 (27.27)*	36.80 (37.35)	45.00 (42.13)	-	-
T_2	Propiconazole 25% EC	0.2%	20.50 (26.96)	25.00 (30.00)	26.37 (30.93)	32.06	41.40
T_3	Carbendazim 50% WP	0.2%	20.37 (26.87)	26.27 (30.83)	29.50 (32.89)	28.61	34.44
T_4	*Trichoderma harzianum*	10^6 cfu/ml	20.13 (26.66)	28.10 (32.01)	31.00 (33.83)	23.64	31.11
T_5	*Bacillus subtilis*	10^6 cfu/ml	20.93 (27.34)	30.34 (33.48)	34.60 (36.04)	17.55	23.11
T_6	*Azardirachta indica* kernel extract	10%	20.42 (26.85)	31.53 (34.16)	35.53 (36.59)	14.32	21.04
T_7	Seed extract of *Annona squamosa*	10%	20.60 (27.02)	33.19 (35.35)	38.35 (38.33)	9.81	14.77
	SEm ±		0.30	0.48	0.41		
	CD at 5% level		NS	1.44	1.22		

Conclusion

Canes growing nursery, fungal diseases cause maximum losses of growth and reduces the good quality seedling production. We showed in this study, among the study Katgal nursery was high in both disease incidence and percent disease index. Mainly canes are affected by leaf spot, leaf blight, root rot and sooty mold diseases and caused by different pathogens like, *Pestolotiopsis oxyanthi*, *Alternaria dianthi*, *Fusarium solani* and *Capnodium salicinum* respectively. Leaf blight diseases was playing major role to limit the quality seedlings production potential in nurseries. In in-vivo evaluations, among the 6 fungitoxicants Propiconazole and Carbendazim @ 0.2 per cent gave effectively control all foliar diseases of canes.

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