Research Article

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Survey and identification of pathogens causing leaf spot disease of arecanut in selected areas of hill zone of Karnataka

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Abstract

Arecanut *(Areca catechu)* is an important plantation crop of India belongs to the family Arecaceae. The arecanut production in Malnad and coastal regions is adversely affected by various biotic and abiotic stresses which causes a drastic reduction in yield. Now a days, among the biotic stresses, leaf spot caused by *Colletotrichum gloeosporioides, Phyllosticta arecae* and *Pestalotia arecae* are becoming more severe. In order to asses the severity of leaf spot diseases an intensive roving survey was carried out during *Kharif*, 2022 in arecanut growing three taluks of Shivamogga and three taluks of Chikkamagaluru district in Karnataka. Among the six taluks surveyed highest disease severity (81.50 %) caused by *Colletotrichum gloeosporioides* was recorded in Adagalale village of Sagara taluk and least disease severity (18.50 %) was recorded in Maloor village of Thirthahalli taluk. Highest disease severity (42.20 %) *Phyllosticta arecae* was recorded in Surakodu village of Sringeri taluk and no disease was recorded in Hilikunji and Nidagodu villages of Hosanagara taluk. Among the six taluks surveyed for leaf spot caused by *Pestalotia arecae,* highest disease severity (62.50 %) was recorded in Karekumbri village of Thirthahalli. The leaf spot causing pathogens were identified based on symptoms and conidial morphological features as *Colletotrichum gloeosporioides, Phyllosticta arecae* and *Pestalotia arecae.*

Keywords : *Arecanut, survey, leaf spot, Colletotrichum, Phyllosticta, Pestalotia, severity*

Introduction

Arecanut *(Areca catechu* L.*)* is an important plantation crop of India. The industry forms the economic backbone of a substantial number of farm families (Balasimha and Rajagopal, 2004). It is extensively used in India by all sections of people as masticatory and in several social and religious ceremonies (Bhat *et al*., 2021). India is the largest producer of arecanut in the world. Presently it is cultivated in 7.31 lakh hectares with a production of 13.52 lakh tonnes and average productivity is 1.84 MT/ha. In India, the major area under cultivation is confined to Karnataka, Kerala and Assam among which, Karnataka stands first in area, production and productivity (Anon., 2020). In Karnataka it is grown in an area of 5 lakh hectares with a production of 10.81 lakh tonnes and productivity of 2.16 MT/ha. Arecanut is affected by a number of diseases at different stages of growth and development. About 20 diseases, causing varying degrees of damages to the palm have been recorded in India (Bavappa, 1982). Among the fungal diseases, leaf spot has became more catastrophic and cause severe yield loss up to 60 per cent (Hedge, 2018).

In recent years, leaf spot has become epidemic in Karnataka and Kerala. Leaf spot of

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arecanut, though considered as a minor disease in the past, has now become a major problem in arecanut cultivation especially during monsoon season. Leaf spot diseases are severe and infect the palms of all ages. They are responsible for destruction of a measurable amount of leaf area and thus reducing the growth rate of the arecanut palm. The leaf spot is reported to be caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Hegde *et al*., 1988) *Pestalotia palmarum* Cooke (Chowdhury, 1946) and *Phyllosticta arecae* Hohnel (Rao, 1964). *Colletotrichum gloeosporioides* was described for the first time by Hegde and Hegde (1986). First report of *Pestalotia palmarum* causing leaf spot disease of arecanut was made by Carl and Bartlett (1922). Höhnel *et al*. (1912) reported *Phyllosticta arecae* on arecanut and its penetration and infection process of conidium on arecanut was revealed by Bhat *et al*. (1983).

The leaf spot disease is generally severe during rainy season. Symptoms of leaf spot appear on the leaves of outer, middle whorls and one to six leaves.

Epidemics caused by *Colletotrichum* species generally occur during rainy, humid, and warm weather, with temperatures ranging between 20°C and 30°C (Shabi and Katan, 1983; Sharma and Kulshrestha, 2015; Kamle and Kumar, 2016). Hence, considering the economic importance of the crop in traditionally cultivated area and reduction in production, a roving survey was undertaken to assess the severity of leaf spot diseases in Shivamogga and Chikkamagaluru districts of Karnataka and also to identify the pathogens associated.

Materials and methods

An intensive roving survey was conducted during *Kharif* 2022 in hilly area of Shivamogga and Chikkamangaluru districts to assess the severity of leaf spot disease of arecanut. Three talukas in each district and in each taluk a minimum of 5 villages were surveyed comprising four fields in each village. In each field five plants were selected randomly and disease severity was assessed using 0 to 5 severity scale (Bhat, 1983) as described in Table 1. Disease assessment was made for each leaf starting from apical spindle leaf to bottom leaf. In each leaf disease assessment was made on one leaflet each at the base, middle and apical portion of leaf. Based on this scale, the percent disease index was calculated using the formula (Wheeler, 1969).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of leaves observed x Maximum score}} \times 100$$

Table 1. Disease score scale for leaf spot of arecanut

Leaf area affected by leaf spots	Disease score	Host response
0 Percent of leaflet covered by leaf spot	0	Immune
1-5 Percent of leaflet covered by leaf spot	1	Resistance
6-15 Percent of leaflet covered by leaf spot	2	Moderate resistance
16-30 Percent of leaflet covered by leaf spot	3	Moderate susceptible
31-50 Percent of leaflet covered by leaf spot	4	Susceptible
>50 Percent of leaflet covered by leaf spot	5	Highly susceptible

For disease incidence on fruit till now there is no standard scale to measure incidence hence we followed 1-6 scale given by Sastry and Hegde (1987) as described in Table 2.

Table 2. Disease rating scale for leaf spot disease on nuts of areca palm.

Grades	Description
1	1-10% nut fall from bunches
2	11-25% nut drop
3	26-50% nut drop
4	51-75% nut drop + spread of the disease to bunch stalk
5	76-100% nut drop + spread of the disease to the main stalk of the bunch
6	Crown death

Based on this scale, the per cent disease index was calculated using the formula (Wheeler, 1969).

Isolation of pathogen

The infected portions along with some healthy parts were cut and surface sterilized using 1 percent sodium hypochlorite solution for 60 seconds. These bits were thoroughly washed in sterile distilled water for three times to remove the traces of sodium hypochlorite if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants and incubated at room temperature (27 ± 1 °C) and observed periodically for fungal growth and sporulation. Colonies, which developed from the bits were identified by microscopic observation considering the mycelial

and spore characters as means for identifying the pathogen. After identification, the cultures were transferred to potato dextrose agar slants and incubated at 27 ± 1 °C for further use (Arunprasad, 2022).

Morphological characterization

For morphological studies of the pathogen, a loopful pure culture of the isolated pathogen from 12 days old culture was placed on the slide and mixed thoroughly with lactophenol to obtain uniform spread. A cover slip was placed over it. Length and breadth of the spores were measured using binocular microscope

Results and Discussion

Data pertaining to survey (Table 3) revealed that, disease severity caused by *Colletotrichum gloeosporioides* ranged from 18.50 to 81.50 per cent. A total of 70 villages were surveyed in Shivamogga and Chikkamagaluru districts. The PDI in Hosanagara taluk ranged from 48.50 (Mudugoppa) to 75.80 per cent (Vatagodu). PDI in Sagara taluk ranged from 62.50 (Kappadury) to 81.50 per cent (Adagalale). Whereas, in Thirthahalli taluk the PDI ranged from 18.50 (Maloor) to 68.50 per cent (Malati) in arecanut orchards. In N. R. Pura taluk, the PDI ranged from 30.50 (Balehonnuru) to 45.58 per cent (Haravari) whereas in Koppa taluka the PDI ranged from 52.20 (Hariharpura) to 71.50 per cent (Shivapura) and in Sringeri taluk disease severity ranged from 52.50 (Ullavalli) to 68.50 per cent (Kaimane and Sringeri town). Among the taluks surveyed to assess the per cent disease severity caused by *Phyllosticta areace,* maximum disease severity (42.20 %) was recorded in Surakodu village of Sringeri and least disease severity (0.00 %) was recorded in Hilikunji and Nidagodu village of Hosanagara taluk. Highest disease severity (62.50 %) of leaf spot caused by *Pestalotia arecae* was recorded in Karekumbri village of Thirthahalli taluk and least disease severity of 10.04 per cent was recorded in Mudugoppa village of Hosanagara taluk.

During roving survey, infection on nut was also observed and the disease severity ranged from 8.50 to 71.85 per cent. Among the different villages surveyed, highest severity (71.85 %) on nuts was recorded in Adagale village of Sagara taluk and least disease severity (8.50 %) was recorded in Lakhmapure village of Thirthahalli taluk and Balehidlu village of N. R. Pura taluk. This is the first attempt to asses the disease severity of *C. gloeosporioides* infecting the nuts under field condition.

It is noticed that, these outbreaks occurred despite the use of chemical management measures, probably due to increasing prevalence of fungicide resistance or changes in population structure or prevalence of favourable weather conditions over a relatively long period of time. The survey results are in agreement with the earlier reports of Naik et al. (2021) where it is reported that Phyllosticta leaf spot to be a common problem in almost all the talukas of Shivamogga district, however it was found to be maximum in Shivamogga taluk (37.6 %) followed by Shikaripura taluk (36.4 %).

Symptomatology of leaf spot disease

During survey the maximum disease severity was noticed on the outermost leaf in the outer whorl and the intensity gradually decreased in the inner leaves. Brown to dark brown or black spots with a broad or narrow halo appear initially on the leaves. These spots get coalesced to form large blighted areas in the advanced stages of infection. Some of these spots showed a central dried greyish portion with dark pycnidia of the fungus on the upper surface of the leaf. The affected palm showed drying and drooping of leaves in the advanced stages. In case of severe infections, the entire crown dried up in seedlings (Fig 1).

Colletotrichum gloeosporioides

The disease appeared as a small round/elliptical light to dark brown spots surrounded by dark brown margins and yellow halo or sunken spots with concentric rings on nuts and in severe cases splitting of nuts was also observed. The results are in agreement with the earlier reports of Hegde and Hegde (1986) and Arunprasad (2022).

Phyllosticta arecae

The typical symptoms appeared as round to oval spot usually isolated, with white and papery centres and dark brown margin. The spots later increased in size and coalesced to form larger lesions (Fig 2). The affected leaves become shredded and disfigured and suffered extensive desiccation. The symptoms were identical to those described earlier by Shukla and Haware, (1972) and Arpitha (2022).

Pestalotia arecae

Characteristic symptoms of leaf spot of arecanut were yellowish or dark brownish spots surrounded by yellow halo. The centers of the spots were brown to black. The spots were more or less circular. In case of heavy infection blightening occurred and withering of infected leaves was observed (Fig 3) and Ahmed (2014) observed similar symptoms caused by *Pestalotia* sp. on beetle vine.

Morphological characterization of leaf spot causing pathogens

Colletotrichum gloeosporioides

On PDA, the pathogen produced dense, cottony, dirty white to greyish mycelium. It produced abundant aerial mycelium at the center of the colony. Later, it produced conidiophores either arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends and with one or two oil globules. Conidia measured 13.01-19.08 µm in length x 3.50- 7.82 µm in width. Conidiophores were simple, filiform measuring 9 to 11.2 µm (Fig 4) and results are similar to the previous work carried out by Pruthviraj (2018) and Arunprasad (2022). Based on the mycelial and spore morphology, fungus under study was identified as *C. gloeosporioides.*

Pestalotia arecae

Colour of the culture on PDA media varied from white to yellow. The growth varied from flat, raised to circular with wavy margins. There was black colour pigmentation in the culture and excellent sporulation was due to the black colour pigmentation. Conidia were five to six celled, of which apical and basal cells were hyaline and three median cells were light brown with varying shades of olive-green colour. Basal appendages were hyaline, straight or slightly curved. There were two apical appendages. The length of conidia varied from 20 to 25.3 µm × 4.2 to 6.3 µm (Fig 4). These morphological and cultural characters of isolated pathogen showed its close identity with *Pestalotiopsis mangiferae* as described by Patel (1988). *Pestalotiopsis mangiferae* produced branched, septate, hyaline mycelium on Richard's agar. Whereas, Kyada (2006) reported that the fungus *Pestalotiopsis guepinii* initially produced cottony white fluffy growth on PDA with hyaline and septate mycelium. The above mentioned results were also in conformity with the work of Selmaoui *et al.* (2014) and Fernadez *et al.* (2015).

Phyllosticta arecae

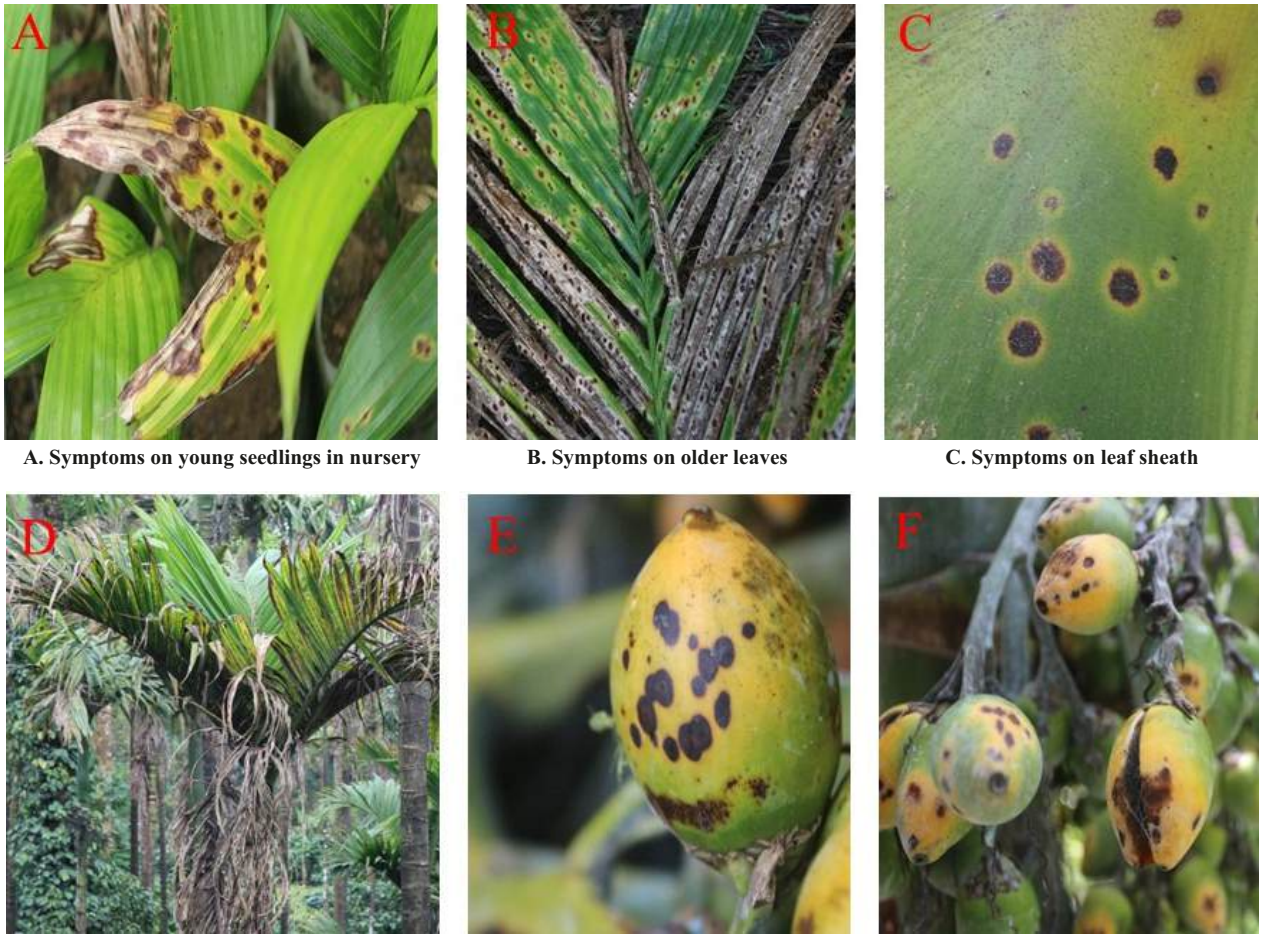
The colony of *Phyllosticta* was initially white, which gradually turned to light to dark brown colour. On PDA medium the pathogen produced olivaceous greenish mycelium. The microscopic observation revealed that the pycnidia were globose to sub-globose with dark brown colour measuring 110.08 µm × 2.23 µm. The pycnidiospores were hyaline, round to oval shaped, monoguttulate measuring 2.51µm × 1.18 µm (Fig 4). The observations on morphological characters of pathogen are in conformity with Barun et. (2017) where *P. zingiberi* causing leaf spot of ginger produced globose to sub globose with dark brown coloured pycnidia and pycnidiospores were hyaline, oval to bullet shaped, monoguttulate.The colony colour exhibited a pattern of light grey at the margins with olivaceous green zonations. Ramakrishnan (1942) also observed the thick growth, dark-olive colouration of the colony of *P. zingiberi.* Zimowska (2013) also observed olive grey aerial mycelium.

Table 3. Survey for assessing the disease severity of leaf spot disease on leaves and nuts

Sl. No.	Taluks		Village	GPS coordinates	Percent disease index (PDI) *Phyllosticta areace*	Percent disease index (PDI) *C. gloeosporioides* on leaves	Percent disease index (PDI) *Pestalotia arecae* on leaves	Percent Disease Index (PDI) *C. gloeosporioides* on nuts
1.	**HOSANAGARA**	1	Hadikai	13.9433096 74.888174	10.3	60.45	15.8	50.6
		2	Mudugoppa	13°48'53.78" 75°2'30.454"	12.4	48.5	10.04	40.8
		3	Hilikunji	13.8270541 75.052944	00	60.5	12.5	58.5
		4	Nidagodu	13.7333446 75.0213694	00	52.5	18.65	48.85
		5	Nagodi	13.9179409 74.8980616	10	68.5	28.75	65.8
		6	Byragodu	13.735146 75.026080	12.5	55.5	18.5	40.8
		7	Mallikoppa	13.78413 74.24513	14.5	55.60	17.5	40.85
		8	Mallekoppa	13.9221 74.9059	8.5	58.65	16.8	51.5
		9	Karkamadi	13.6928 79.5893	12.5	59.82	24.8	56.52
		10	Chakranagara	13.8115276 74.9853517	10.5	62.5	25.6	58.5
		11	Vatagodu	13°81' 52.067" 74°98' 38.038"	20.5	75.80	35.85	68.50
		12	Hulukoppa	137163022 750489824	18.5	66.5	28.35	52.85
				Mean	**10.85**	**60.40**	**21.12**	**52.83**
2.	**SAGARA**	13	Kappadur	13.9385812 74.8709511	25.8	62.50	22.50	55.48
		14	Hudikesari	13.927985' 74.838907	14.5	80.20	24.80	54.80
		15	Byadagodu	13.918564 74.836929	24.84	78.50	42.50	65.70
		16	Adagalale	74.826113 13.949571	18.5	81.50	48. 50	71.85
		17	Kanchikere	74.874562 13.784518	16.50	74.58	38.40	68.50
		18	Tumari	14.023428 74.854706	25.80	72.56	35.50	65.80
				Mean	**20.99**	**74.97**	**32.74**	**63.68**
3.	**THIRTHAHALLI**	19	Kotikoppa	13.7565505 75.2232938	15.50	45.50	25.80	38.50
		20	Lakshimipura	13.6159936 75.2676485	8.50	21.50	20.50	15.60
		21	Maloor	13.618950 75.275527	12.50	18.50	16.80	14.80
		22	Lakhmapure	13.61434 75.267471	10.50	20.50	15.60	8.50
		23	Talluru	13.5177184 75.1216297	25.60	62.58	28.30	58.50
		24	Karekumbri	13.518231 75.12619	18.50	55.50	32.50	45.50
		25	Malali	13.5257930 75.1390738	22.50	58.25	45.00	52.50
		26	Guddekoppa	13.9343 75.4948	15.50	42.50	35.20	35.20
		27	Kodlu	13.6498389 75.1789187	12.50	52.50	35.20	48.50

		28	Horabylu	13.8111685 75.2361318	8.50	48.50	38.20	38.50
		29	Nonaburu	13.7894583 75.1919036	10.50	45.20	28.50	38.50
		30	Sampagalu	13.81116 75.23613	8.50	50.50	18.50	42.50
		31	Balagatte	13.64077 75.27466	14.50	42.50	25.50	35.20
		32	Basageri	13.629942 75.177353	12.20	38.50	31.20	25.20
		33	Kodlu	13.6476 75.1806	15.50	48.44	28.50	32.20
		34	Jambetalluru	13.777392 75.237879	8.5	35.20	18.50	24.50
		35	Kandaka	13.778167 75.195239	12.50	38.50	24.60	15.50
		36	Handalasu	13.5407151 75.1291271	24.20	52.30	28.50	46.50
		37	Karekumbri	13.521425 75.1214775	28.50	65.20	62.50	59.25
		38	Yadadalu	13.4001 75.2100	14.20	45.20	24.50	38.50
		39	Ulavalli	13.54752 75.25741	16.50	46.50	25.60	38.50
		40	Sampagara	13.578589 75.412155	12.20	47.20	25.20	42.20
		41	Kalmane	14.122340 75.35898	12.50	52.20	30.00	45.20
		42	Hondalase	13.912633 75.573969	8.50	55.50	24.20	45.60
		43	Malati	13.506318 75.091337	8.50	68.50	45.80	58.50
				Mean	**14.29**	**46.29**	**29.38**	**37.75**
4.	**N. R. PURA**	44	Haravari	13.5042123 75.4225303	18.50	35.50	52.70	12.50
		45	Balehonnur	13.50255265 75.4279957	15.50	30.50	45.50	12.50
		46	Haravari	13.486128 75.438264	12.50	45.58	48.50	10.00
		47	Sankse	13.5430450 75.4653578	10.52	38.50	45.62	12.50
		48	Balehidlu	13.352873 75.468113	8.50	35.20	38.50	8.50
		49	Kanoor	13.31262 75.25583	10.52	38.50	20.50	15.50
				Mean	**12.67**	**37.29**	**41.88**	**11.91**
5.	**KOPPA**	50	Ammadi	13.547499 75.348058	25.50	55.50	16.50	40.50
		51	Shettyadlu	13.3446 75.59210	15.20	58.50	18.50	45.20
		52	Harandur	13.533209 75.382431	28.50	62.50	32.50	52.50
		53	Thalmakki	13.324943 75242940	15.50	55.80	35.50	52.50
		54	Haranduru	13.54481 75.381836	32.50	68.50	25.50	58.50
		55	Shivapura	13.554783 75.339111	12.50	71.50	35.20	47.50
		56	Suruli	13.505942 75.298189	18.50	58.50	28.50	35.20
		57	Hariharpura	13.522036 75.301194	22.20	52.20	36.80	26.50
				Mean	**21.30**	**60.37**	**28.62**	**44.80**
6.	**SRINGERI**	58	Miginakallu	13.445296 75.258704	28.55	65.20	41.50	48.28

59	Torahadlu	13.532072 75.371009	22.50	55.20	35.60	43.20
60	Anagonda	13.936005 75.258794	31.20	58.50	38.50	48.50
61	Kavadi	13.2652 75.1612	24.50	62.50	48.50	50.50
62	Addagadde	13.2653 75.1612	22.50	54.20	38.50	42.20
63	Kaimane	13.444890 75.237325	28.50	68.50	31.50	61.50
64	Sringeri town	13.423223 75.250662	38.20	68.50	42.50	52.20
65	Korankudige	13.416033 75.228869	35.20	58.20	38.50	51.20
66	Torahadlu	13.2727 75.1637	28.50	64.20	38.50	55.40
67	Gubbagodu	13.418402 75.225857	35.20	54.20	45.20	27.20
68	Anegunda	13.418887 75.234237	38.20	68.20	36.58	45.20
69	Surakodu	13.408853 75.2184035	42.20	61.20	44.58	32.20
70	Ullavalli	13.418368 75.234231	38.50	52.50	35.20	35.20
		Mean	**31.82**	**60.85**	**39.62**	**45.59**



A. Symptoms on young seedlings in nursery **B. Symptoms on older leaves** **C. Symptoms on leaf sheath**

D. Symptoms on aged palms **E and F Brownish to blackish spots on nuts**

Fig.1. Symptoms of *Colletotrichum gloeosporioides* on leaves and nuts of arecanut

Fig. 2. Symptoms of *Phyllosticta areace* on arecanut leaves

Fig. 3. Symptoms of *Pestalotia areace* on arecanut leaves

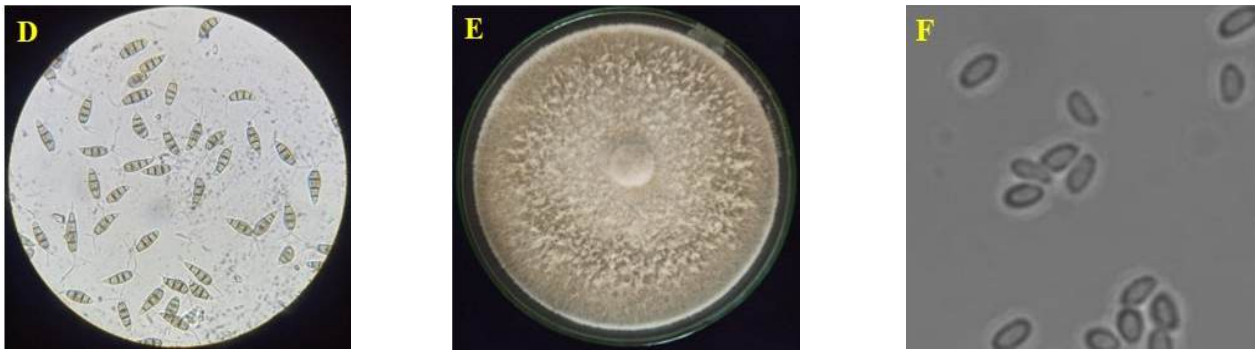


Fig. 4. Pure culture and conidia of arecanut leaf spot causing pathogens

A and B. Pure culture and conidia of *Colletotrichum gloeosporioides*

C and D. Pure culture and conidia of *Pestalotia arecae*

E and F. Pure culture and conidia of *Phyllosticta arecae*

Conclusion

Leaf spot of arecanut was found to be a highly destructive disease in hill zone of Karnataka which was revealed by survey result. Based on symptoms and isolation from suspected leaf samples it is revealed that *Colletotrichum gloeosporioides, Pestalotia arecae* and *Phyllosticta areca* were found to be associated with leaf spot disease of arecanut palm. Survey study at six talukas indicated that high disease severity was found in Nagodi and Chakranagara (Hosanagara) leads to complete failure of crop followed by Sagara, Koppa, Sringeri and Thirthahalli. This might be due to due to inoculum abundance resulting from previous outbreak, high temperature, high humidity and wind speed. Therefore, proper cultural practices and phytosanitary measures and prophylactic fungicides spray should be followed by farmers to overcome the problem.

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