ANTHRACNOSE DISEASE OF *CARISSA CARANDAS* LINN. CAUSED BY *COLLETOTRICHUM INAMDARII*

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Introduction

Carissa carandas L., popularly known as 'Karaunda', is cultivated in various South-East Asian countries for its fruits and fencing.

So far *Pestalotia versicolor* is the only fungal parasite reported on this plant (Mundkur, 1942). The present paper describes an anthracnose disease caused by a species of *Colletotrichum*. The disease seems to be very widely distributed in various parts of Uttar Pradesh. At Banaras all the hedges of 'Karaunda' are seriously infected by it.

Material and Methods

Material for the study was obtained from various places in Uttar Pradesh. The organism was isolated from the affected tissue and purified by single spore culture. Hand sections were stained in cotton blue in lactophenol. For microtome sections the materials were fixed in Karpenchenko's solution and the sections were stained in Light Green and Magdala Red.

Symptoms

Pinkish red spots of the size of pinheads mainly confined to the leaves are the first indications of the disease. These spots increase in size and may spread over the greater part of the leaves. Lesions are irregular, later turn brown and are surrounded by a red margin which passes into the green colour of the leaf through a yellow halo. Sooner or later the central region turns grey in which minute scattered black dots representing the fructifications of the fungus are developed. In very severe cases the affected leaves dry up. In combination with *Pestalotia versicolor* this disease produces conspicuous dieback symtoms)Fig. 1).

Pathogenicity

Experiments were carried out on seedlings or on tender twigs kept in water. Inoculations were made in moist glass chambers on both surfaces of the leaf with or without injury using spore suspension or mycelial growth.

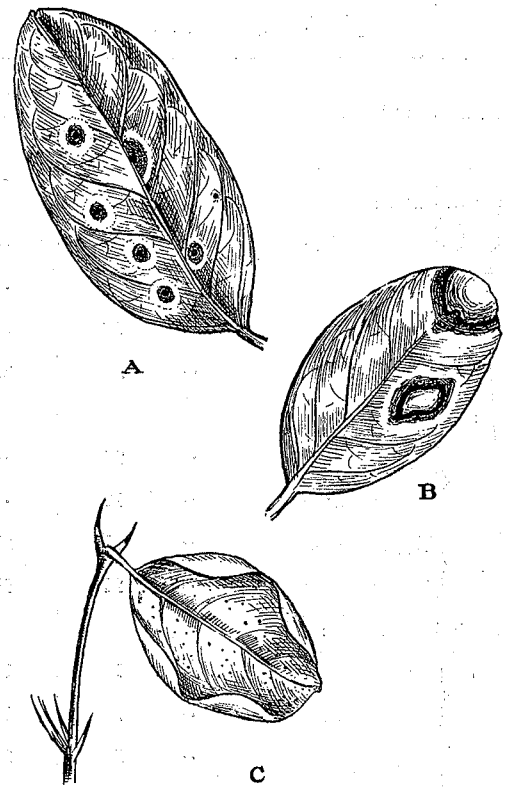


FIG. 1.—A & B. Different stages in the development of the disease.
C. The last stage showing 'die-back'.

It is obvious from Table I that infection occurs only after injury showing that the parasite is purely a wound parasite. The severity of the disease can be explained by the fact that bushes being thorny, the leaves are injured in windy weather. Spore suspension or mycelial inoculum are found to be equally effective on both the surfaces of the leaves irrespective of the presence or absence of stomata. Inoculum in leaf extract is able to infect uninjured leaves as well.

As infection occurs generally after injury the exudations of the plant seem to provide stimulation to the spores which become able to infect even the uninjured portions of the leaves in nature.

Cross inoculation experiments have indicated that neither the anthracnose of *Carissa carandas* is able to infect *Citrus medica* and cucumber nor the anthracnose of the above 2 plants is able to infect *Carissa carandas*. The fungus on *Carissa carandas* is therefore a different one and has been named *Colletotrichum inamdarii* after Prof. R. S. Inamdar, the teacher of the senior author.

TABLE I

Infection Experiments

Treatment of leaves			No. of leaves inoculated	No. of leaves infected	Percentage of infection
1 Spore suspension in water :					
(i) Uninjured	Upper surface	..	25	..	..
	Lower surface	..	20	..	..
(ii) Injured	Upper surface	..	26	26	100
	Lower surface	..	26	26	100
2 Mycelial growth :					
(i) Uninjured	Upper surface	..	23	..	..
	Lower surface	..	19	..	..
(ii) Injured	Upper surface	..	21	21	100
	Lower surface	..	21	21	100
3 Spore suspension in leaf extract :					
(i) Uninjured	Upper surface	..	19	18	95
	Lower surface	..	20	19	95
(ii) Injured	Upper surface	..	25	25	100
	Lower surface	..	15	15	100

MORPHOLOGY

The mycelium is localised in the diseased spots. It is thick, septate. branched and intracellular. When young the hyphæ are hyaline with granular protoplasm and no oil globules but when old they become thick-walled, brownish, septate, profusely branched with irregular swellings filled with granular substance containing oil globules. Thickness of hyphæ in the host cell varies from 1·4μ to 4·15 μ and in culture from 2 μ to 4·85 μ.

Acervuli occur on both the surfaces of the leaf and are visible to the naked eye as black dots.

In the formation of acervulus the mycelium collects to form a well-defined stroma of thick-walled cells tightly pressed together. The sides of the stroma are slightly raised to form a shallow grooved acervulus. The young acervulus is covered by the host tissue being slightly raised above the general surface. By the continued growth of conidia

and conidiophores the overlying tissue is ruptured and the acervulus is left open. Acervuli are bordered by black septate setæ (Fig. 2).

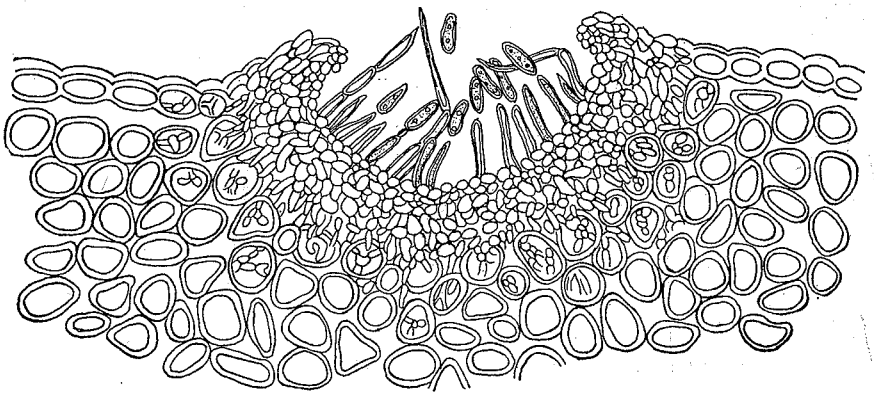


FIG. 2. Acervulus in section, showing setæ, conidia and conidiophore. Mycelium is intracellular.

Conidiophores are cylindrical, septate, unbranched projections arising from the surface of the stroma and filled with granular protoplasm.

Conidia are unicellular but become two celled before germination, oblong broadly rounded at the apices. They are hyaline individually but pinkish in mass embedded in gelatinous substance. The conidia contain two to several oil globules.

Setæ have been noted in culture as well as in nature. They are sterile hyphæ arising from the margin of the acervuli, thick-walled, septate, darker and longer than the conidiophores and pointed at the ends.

TABLE II

Size of various reproductive structures (μ) on the host and culture medium

	Structure	Host	Culture media
1	Acervulus ..	69·25 to 204·45	176·19 to 545·20
2	Conidiophore ..	5·54 to 11·08	22·16 to 36·55
3	Conidia ..	11·12 to 26·41 by 2·98 to 5·56	
4	Setæ ..	30·47	55·4 to 77·56

DESCRIPTION OF THE SPECIES

Colletotrichum inamdarii sp. nov. spots confined to leaves, minute, deep pink with yellow halo, enlarging into irregular lesions. Lesions

have grey central region, bounded by shades of deep brown and pink with prominent yellow halo. Acervuli black, scattered in the grey central region, on both the surfaces, erumpent, setose and measure 69 μ to 204 μ average 110 μ in diameter. Conidiophores non-septate, hyaline, cylindrical, slightly tapering towards the distal end measuring 2·4 μ by 5·5–11·0 μ. Conidia, hyaline bacilliform, straight, stout, thin-walled, non-septate with two or more refractile globules measuring 11·0 to 26·0 μ by 2·98 μ to 5·56 μ.

Conidia become bicelled before germination. Setæ distributed irregularly, tapering towards the distal end, septate, black, brown measure 30·5 μ average. Stroma subepidermal, mycelium intercellular hyaline to light brown, measures 1·4 to 4·2 μ thick.

Habit.—On the leaves of *Carissa carandas*, L Banaras. Coll. Akshaibar Lal (August, 1945) type. The type specimen is deposited in the Herb. Crypt. Ind. Orient., New Delhi.

Latin Translation: *Colletotrichum inamdarii* Lal, spec. nov.

Maculæ in foliis tantum, minutæ, profunde roseæ, nimbo luteolo, mutantes in læsiones irregulares. Læsiones griseæ in regione centrali, circumdatæ tinctione alte brunnea et rosea, nimbo prominenti luteo. Acervuli nigri, dispersi in regione grisea centrali, in utraque pagina foliorum, erumpentes, setosi, magnitudinis 70 to 20 μ (110 μ) in diam. Conidiophori haud septati, hyalini, cylindrici, tenuiter fastigiati in apice semoto, magnit. 5·54 μ ad 11·0 μ longitudine. Conidia hyalina, bacilliformia, recta, valida, parietibus tenuis prædita, haud septata, duobus vel pluribus globulis ornata, magnit. 11 to 26 μ × 2·9 to 5·56 μ. Conidia evadunt bicellulata ante germinationem. Setæ irregulariter dispersæ, fastigiatæ in apicem remotum, septatæ, nigrobrunneæ, magnit. 30·5 μ longitudine in medietate. Mycelium intracellulare hyalinum ad tenuiter brunneum, magnit. 1·4 to 4·2 μ crassitudine.

Typus lectus in foliis *Carissæ carandas* Linn., in loco Banaras ab Akshaiberlal, *mense augusto* 1955.

Hibernation

Spores are short lived and die within 1½ months' time. The organism has been found to hibernate through the mycelium in the tissue and become active in the next favourable season after the first rain and start the disease.

Germination of Conidia

Studies in hanging drop culture show that conidia germinate in sterile distilled water in 6–12 hours. Germ tube is formed at one end or at both the ends or even from the middle. Ordinarily one of the oil globules migrates to each of the daughter cells formed by septation. In the germ tube the oil globule breaks up into smaller ones and gradually disappears. The germ tube when it touches a hard surface swells up and a partition separates this swollen region where the oil globules reappear. The wall thickens, turns brown and the smaller oil globules

fuse together to form one prominent globule. The free end of the germ tube which does not touch the glass furface instead of producing appressoria, produces typical conidia. On hard surface like glass, there is no further development of the appressoria in distilled water, but in host extract suspension 80% of appressoria develop normal infection hypha after about 2½ hours at 22° C. During germination the single refractive body of appressorium breaks up into smaller ones. The wall of the appressorium ruptures and a fine germ tube develops at any point of the appressorium which elongates into infection hypha (Fig. 3).

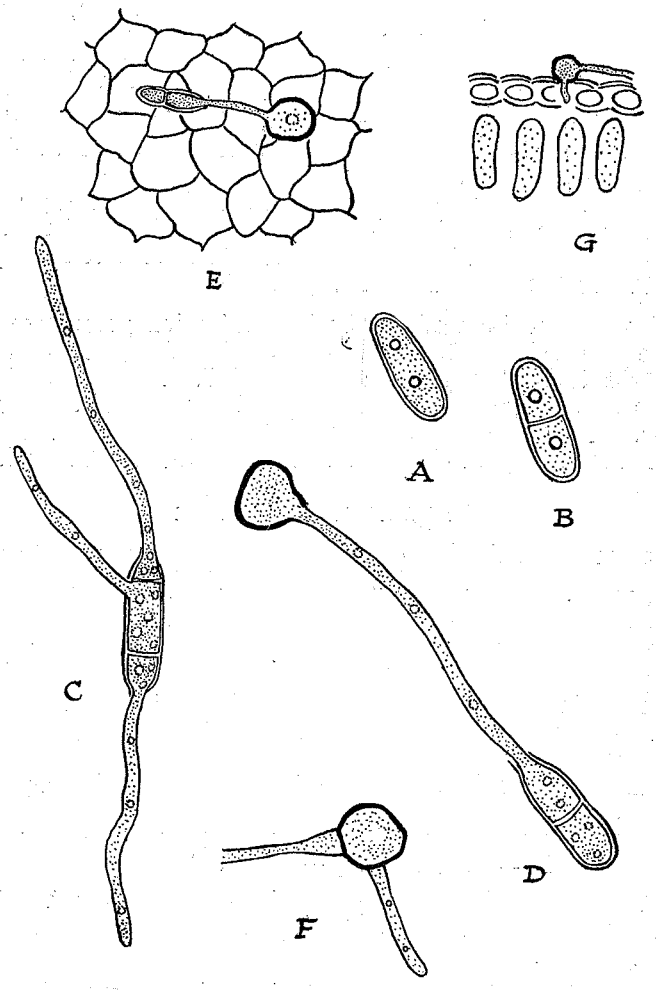


FIG. 3.—A. Conidium. B. Conidium before germination. C. Germtube formation. D & E. Formation of appressorium. F & G. Formation of infection hypha.

Spore suspensions in leaf juice and distilled water were also sprayed over the leaves which were incubated under bell jar in moist condition. On the host conidia germinate to form appressoria in both the cases but it is only in the case of spore suspension in leaf juice that appressoria develop infection hyphæ which penetrate the leaf. Dey (1919, 1933) also noted in the case of *Colletotrichum lindemuthianum* and *C. gleosporioides*, that the conidia germinate to form appressoria but the infection hyphæ do not develop unless there is a stimulation by the secretion of the host.

5·5% sucrose solution has been found to be the best for germination as it gives 87·8% germination after 11 hours as against 22% in sterile distilled water and 71·5% in host leaf extract. The germ tube length is also greatest (60·1 μ) in sucrose solution as against 45·5 μ in host leaf extract and 16·6 μ in sterile distilled water.

Temperature relation was studied by keeping hanging drop cultures at various temperatures ranging from 20° C. to 37° C. With increasing temperature the percentage of germination as also the length of the germ tube increase and reach their optimum (43·5%; 25 μ) at 30° C. after which there is a decrease. The conidia resist a temperature of 48° C. for 10 minutes but are unable to survive a temperature of 49° C. for the same duration.

pH of the medium affects both germination percentage and the length of the tube. The pH range suitable for germination varies widely (pH 4–10), the optimum being pH 7. Below and above this both the percentage of germination and the length of germ tubes decrease. At pH 3 and 11, germination stops altogether. Relatively, alkaline reactions (pH 8–11) seem to be more suitable than acidic reactions.

Growth and Nutrition

The growth of the fungus was studied on various solid and liquid media. All the media used were found suitable for the growth of the fungus. The fungus showed greater liking for such media as Richards' solution, potato dextrose and turnip juice both in solid and liquid form as compared to the host extract and Brown's starch.

Growth in various media seems to be mainly governed by the amount of sugar in the medium. Dry weight is greatest (2·432 gm.) in Richard's solution as it contains 5% sugar whereas it is least (0·98 gm.) in Brown's solution which contains only 0·2% sugar.

Relative growth rate has been calculated acording to the formula

$$R = 100 (\text{Log}_e W_2 - \text{Log}_e W_1)$$

where R represents the rate of growth in percentage and W_1 the initial growth in term of dry weight of the mycelium and W_2 the final weight of the mycelium.

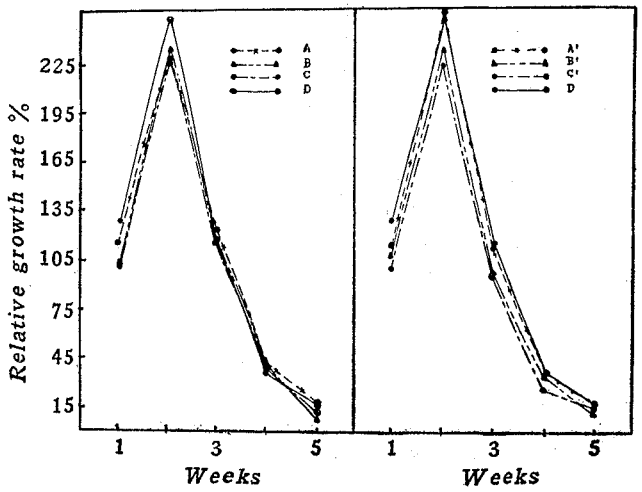
In general, relative growth rate (Fig. 4) increases till it reaches an optimum between second and third week, thereafter there is a sudden fall. The sudden fall in the growth rate seems to be due to the rapid

utilization of some ingredients of the nutrient medium after which the growth rate slows down.

TABLE III

Growth on different liquid media
Temperature 18–20° *C.*

Medium	Dry wt. of the fungus in gm.				
	1	2	3	4	5 weeks
Richard's solution ..	0·036	0·458	1·424	2·051	2·432
Turnip juice ..	0·031	0·405	1·224	1·752	2·075
Potato dextrose solution ..	0·032	0·316	1·087	1·654	1·953
Host twig extract ..	0·028	0·295	0·952	1·442	1·525
Host leaf extract ..	0·028	0·275	0·875	1·275	1·427
Brown's starch solution ..	0·029	0·300	0·789	1·110	1·238
Brown's solution ..	0·027	0·256	0·653	0·846	0·98



A—Potato Dextrose
B—Host twig extract
C—Host leaf extract
D—Richard's solution

A'—Turnip juice
B'—Brown's starch solution
C'—Brown's solution
D —Richard's solution

FIG. 4. Relative growth rate of *Colletotrichum inamdarii* in different liquid media

It has been observed that with the increase in the depth of the medium, growth increases but the fall in the growth rate with time is

greater with the increase in the depth of the medium. This may be casued by the greater production of staling substances with prolonged growth.

The growth of the fungus is greater in alternate light and darkness (88 mm. in diameter) than either continuous light (80 mm. in diameter) or continuous darkness (83·5 mm. in diameter). However, growth in darkness is greater than in light.

Growth of the fungus increases with the increase in temperature of incubation with an optimum at 28°C. after which decrease in the growth results with further increase in temperature.

The cardinal points of pH for growth of the fungus are exactly the same as for the germination of conidia. It should, however, be noted that the fungus whether grown in alkaline or in acidic solution alters the pH value of the medium to neutrality (pH 7).

Constituents of the Medium

It has been found that sugar is the most important constituent for the fungus. Growth is meagre (0·27 gm.*) without sugar in the medium as compared to the control (1·358 gm.*). Of the various carbohydrates, sucrose, glucose, maltose, galactose, levulose and lactose, sucrose is the most suitable form. But other forms of sugar are also used fairly well. Phosphate is another important constituent without which growth is very poor. Next in importance is nitrate. The most suitable form of nitrogen has been found to be sodium nitrate (0·968 gm.*) but other inorganic forms of nitrogen such as ammonium nitrate (0·705 gm.*), ammonium sulphate (0·622 gm.*), and ammonium chloride (0·412 gm.*) are all fairly well utilised. Organic forms of nitrogen such as asparagine (0·915 gm.*) and urea (0·85 gm.*) are better than inorganic forms except sodium nitrate. Sodium nitrate depresses the growth.

Enzymes

Diastase, invertase, protease and laccase have been detected as intracellular and extracellular enzymes following the method of Nutman (1929).

Control

Of the toxic substances tested, 0·1% copper sulphate in aqueous solutions, has been found to check the growth of the mycelium and the germination of the spores. Copper sulphate sprays therefore can be used effectively in cases of severe infection.

Summary

1. A leaf-spot disease of *Carissa carandas* caused by a new species of *Colletotrichum* has been described and named as *Colletotrichum inamdarii* sp. nov.

* Figures indicate the dry weight of the mycelium after 21 days' growth.

2. Pathogenicity experiments have indicated that although a wound parasite it behaves as a normal parasite in host suspension. In nature, exudation from wounds caused by thorns provides stimulation for normal infection.

3. The fungus hibernates in the unfavourable season in the form of the mycelium.

4. Germination of conidia and their method of infection has been described.

5. Physiology and nutrition of the fungus have been studied. Best growth occurs at 27° C. and at neutral reaction of the medium. Sucrose is the best form of sugar and sodium nitrate is the best source of nitrogen.

6. Copper sulphate sprays have been suggested for the control of the disease.

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