AN *ALTERNARIA* DISEASE OF SAFFLOWER*

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INTRODUCTION

SAFFLOWER (*Carthamus tinctorius* L.), an annual herbaceous plant with large orange-coloured flower heads, is grown in many parts of the world, Southern Europe, Egypt, Persia, India, China, Southern Rhodesia and South America. In India it is cultivated in Northern, Eastern, Central and Western India for its florets which are the source of a reddish dye, *carthamin*, and for its seeds from which an oil of considerable commercial importance is extracted. Safflower seed cake, a by-product during the extraction of oil, is used as a fertilizer.

In 1936 a leaf-spot disease due to a species of *Alternaria* was noticed in this crop in the Botanical Section of the Imperial Agricultural Research Institute at Pusa. It was common in the cultivators' fields at Pusa, Samastipur, Dharbhanga, Patna and Muzaffarpur wherefrom specimens were obtained and examined. It has been reported from the Central Provinces[1] but is evidently unknown in any other part of the country.

The extent of damage caused by it appears, however, to be very slight.

SYMPTOMS OF THE DISEASE

The disease first makes its appearance just before flowering and is manifest on all parts of the plant especially the leaves.

In the beginning minute brown to dark brown spots, one to two millimetres in diameter, with concentric rings appear on the leaves. The diameter of the spots gradually increases to about one centimetre. Very often two or more adjacent spots coalesce and form large irregular lesions. The spots gradually become darker on account of the formation of the fructification. The central portion of the spot is generally light brown and is surrounded by a number of dark rings alternating with light ones. With the maturing of the spots, shot holes appear in the infected areas and if the whole leaf is attacked the blade breaks in an irregular manner due to the brittleness of the dead tissue.

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* Major portion of the work was carried out by the author in the Mycology Section of the Imperial Agricultural Research Institute.

[1] Private communication from the Mycologist, Central Provinces.

The disease is less severe on the stem and petiole where the spots are elongated. If the parasite attacks the flower buds they fail to open. Minute dark brown spots first appear at the base of the calyx ; these spots enlarge, spread and later attack other parts of the flower. The unopened flowers shrivel and dry up. Fig. 2 shows the symptoms of the disease.

Morphology of the Parasite on the Host

Mycelium.—The mycelium of the organism in the tissues of the infected area is septate and inter- and intra-cellular, with slight constrictions at the septa. The hyphæ, when young, are sub-hyaline, narrow and sparsely septate but when mature they are dark coloured, more frequently septate and broader.

Conidiophore.—The conidiophores are formed on the central dead portion of the spots. They are stout, erect, rigid, unbranched, septate and slightly constricted at the septa. They arise singly or in clusters bursting the epidermis or through the stomata, and are brown to olivaceous in colour (except the tip which is almost hyaline), about 6 to 10 μ in width, septate (the number of septa varying from 0 to 5) and rounded at the tip which is marked by a single terminal scar ; a lateral scar is sometimes also visible. The length ranges from 15 to 85 μ. Sometimes a spherical swelling (upto 12 μ in diameter) is seen at the base of the conidiophore.

Conidia.—The conidia (Fig. 1) are irregular in shape, with an apex, which is usually blunt, though tapering. Some of the smaller spores are roughly spherical and others elongate-cylindrical with rounded ends. The basal scar is usually plainly visible as also a definite

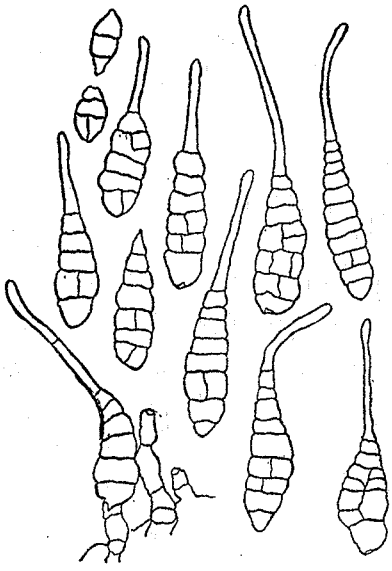

Fig. 1. Conidia of *Alternaria carthami* from nature (× 520).

apical scar, showing that a chain of at least two spores must have occurred. The conidia are light brown, translucent and a majority of them possess a long beak. They measure 36 to 171 μ with the beak, and 36 to 99μ without the beak, in length, and 12 to 28 μ in width. The conidia are usually 3 to 11-celled ; longitudinal septa are common, their number has been found to vary from 0 to 6. The beak is very lightly brown near the base and almost hyaline at the apex ; it measures 15 to 84 μ in length and 3 to 5 μ in width. A few spores without any beak sometimes occur. The surface of the conidia is smooth but sometimes with a granular appearance is surface view.

Isolation and Infection Experiments

The infected parts always showed the presence of olivaceous brown mycelium and spores of *Alternaria* species. Several single spore isolations were made and all isolations were found to be identical.

(i) *Inoculation on the Host.*—Inoculation experiments were performed on plants which had been grown from sterilized seeds. Two different methods of inoculation were followed : plants were either sprayed with spore suspension in sterile water or after spraying the plants with sterile water masses of culture containing spores and mycelium were placed on the required spots with a sterilized needle. The plants were kept covered by bell-jars for 24 or 48 hours and kept moist by occasional spraying with sterile water.

In another series, plants were inoculated by both the methods described above but they were not covered with bell-jars. The results of the inoculation experiments are summarised in Table I.

TABLE I

Summary of the results of inoculation experiments on Safflower by Alternaria *sp.*

Method of inoculation	Whether covered or not by bell-jars	No. of plants inoculated	No. of plants infected	No. of control plants	Controls infected
Mycelium and spores ..	Covered	72	70	32	Nil
Do. ..	,,	60	60	28	,,
Do. ..	Not covered	42	14	19	,,
Do. ..	,,	27	9	12	,,
Spore suspension ..	Covered	67	65	32	,,
Do. ..	,,	74	70	31	,,
Do. ..	Not covered	28	7	10	,,
Do. ..	,,	37	12	17	,,

It will be observed from the data presented in Table I that the presence of moisture is essential for infection ; very few plants took infection when they were not covered by bell-jars even though they were sprayed with sterile water from time to time. The method of inoculation made no difference since infection readily occurred whether

the inoculum was applied as a spore suspension or as a mass of mycelium and spores. The fungus was in every case reisolated from the infected plants. Controls were kept but in no case did they become infected.

(ii) *Cross Inoculations.*—Cross inoculation experiments were carried out on hosts which are common hosts of *Alternaria* sp. The results are recorded in Table II.

TABLE II

Cross inoculation experiments with safflower Alternaria

Hosts	No of plants inoculated	No of plants took infection
Potato (*Solanum tuberosum* L.)	27	Nil
Tomato (*Lycopersicum esculentum* Mill.) ..	17	,,
Cucumber (*Cucumis stativus* L.)	15	,,
Cotton (*Gossypium herbaceum* L.)	29	,,

Data presented in Table II show that the species of *Alternaria* isolated from safflower does not infect potato, tomato, cucumber and cotton plants.

GROWTH IN CULTURE

The fungus was grown on oat meal agar, Hopkin's agar, Dox's agar, potato-dextrose agar, Brown's standard synthetic agar and malt extract agar at room temperature (28—30° C.). The linear rate of growth was practically the same in the first four media ; it was slightly less in malt-extract agar and the least in Brown's standard synthetic agar.

In all the media in which the fungus was cultivated there was very little aerial mycelium. Mycelial growth was mostly submerged. Sometimes a tuft of floccose dark green aerial mycelium was seen around and about the inoculum. The submerged mycelium also gave the media a dark green colour. The colour around the colony was mostly white except in the potato-dextrose agar where it showed a slightly yellowish tint.

TEMPERATURE RELATIONSHIP

The linear rate of growth of the safflower *Alternaria* was studied on Hopkin's agar at various temperatures. The experiment was carried out in selected petri-dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the colonies after seven days growth are presented in Table III.

TABLE III

Growth of safflower Alternaria *at various temperatures*

Temperature (° C.)	Diameter of the colonies (mm.)
15	24
20	26
25	42
30	45
35	6

It will be observed from the data presented in Table III that the optimum temperature for growth lies between 25° and 30° C.

HYDROGEN-ION CONCENTRATION

Richards' solution as modified by Karrer and Webb (1920) was used for studying the growth rate of the fungus at different hydrogen-ion concentrations. 30 c.c. of the solution together with the required amount of N/5 acid and N/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (1917). Four flasks were prepared for each pH value. The flasks were inoculated with a young growing culture of the fungus and incubated at room temperature (28—30° C.). After 30 days the dry weight of the mycelium was determined and is shown in Table IV.

TABLE IV

Growth of safflower Alternaria *in Richards' solution at different hydrogen-ion concentrations*

Hydrogen-ion concentration	Dry weight (mg.)
3	121
4	176
5	207
6	478
7	369
8	292
9·2	145

From the data presented in Table IV it will appear that the fungus can grow in a wide range of hydrogen-ion concentrations. The range of optimum reaction appears to lie between pH 6 and 7 and the amount of mycelium produced is the greatest at pH 6.

IDENTIFICATION OF THE PARASITE

Hitherto no species of *Alternaria* has been reported on *Carthamus tinctorius*. From a consideration of the morphology of the spore and the beak the nearest species to safflower *Alternaria* have been found

to be *Alternaria solani* (Ell. & Mart.) Jones & Grout, *A. tomato* (Cooke) Weber, *A. cucumerina* (E. & E.) Elliot and *A. macrospora* Zimm. But from the reported spore measurement data presented in Table V it will become clear that the *Alternaria* under study does not agree with any one of them.

TABLE V

Comparison of spore measurement data of safflower Alternaria *with other related species of the genus*

Fungus	Length (μ)		Total length including beak	Width (μ)
	Conidial body	Beak		
A. solani (Ell. & Mart.) Jones & Grout.	..	..	120–296	12–20
A. tomato (Cooke) Weber ..	..	..	100– 20	20–22
A. cucumerina (E. & E.) Elliot ..	30–75	25–35	55–110	15–25
A. macrospora Zimm.	..	..	150– 70	20
Safflower *Alternaria*	36–99	12–84	36–171	12–28

Some diseased leaves of safflower and a culture of the *Alternaria* sp. were sent to Dr. S. P. Wiltshire, Director, Imperial Mycological Institute, Kew, England. According to him the fungus came nearest to *A. macrospora*, *A. tomato*, *A. solani* and *A. cucumerina* but he concluded that the safflower fungus does not exactly match with any one of them.

Cross inoculation experiments carried out have shown that the *Alternaria* on safflower does not attack potato, tomato, cotton and cucumber plants. This together with the spore measurements seems to justify the establishing of this fungus as a new species for which the following name is suggested :

Alternaria carthami CHOWDHURY SP. NOV.

Alternaria carthami sp. nov.—Vegetative hyphæ septate, inter- and intra-cellular, when young sub-hyaline, narrow, sparsely septate, but when mature dark coloured, more frequently septate, broader. Conidiophores stout, erect, rigid, unbranched, septate, slightly constricted at the septa, arising singly or in clusters bursting the epidermis or through the stomata, brown to olivaceous in colour, 6 to 10 μ in width. Conidia light brown and translucent, muriform, formed at the tips of the conidiophores singly or in chains, 3 to 11-celled, longitudinal septa few, usually possessing a long beak ; conidia measure 36 to 99 μ $\times$12 to 28 μ without the beak. Beak very lightly brown near the base and almost hyaline at the apex, filiform, measuring 12 to 84 μ $\times$3 to 5 μ.

Habitat.—Parasitic on the leaves and stems of *Carthamus tinctorius* at Pusa.

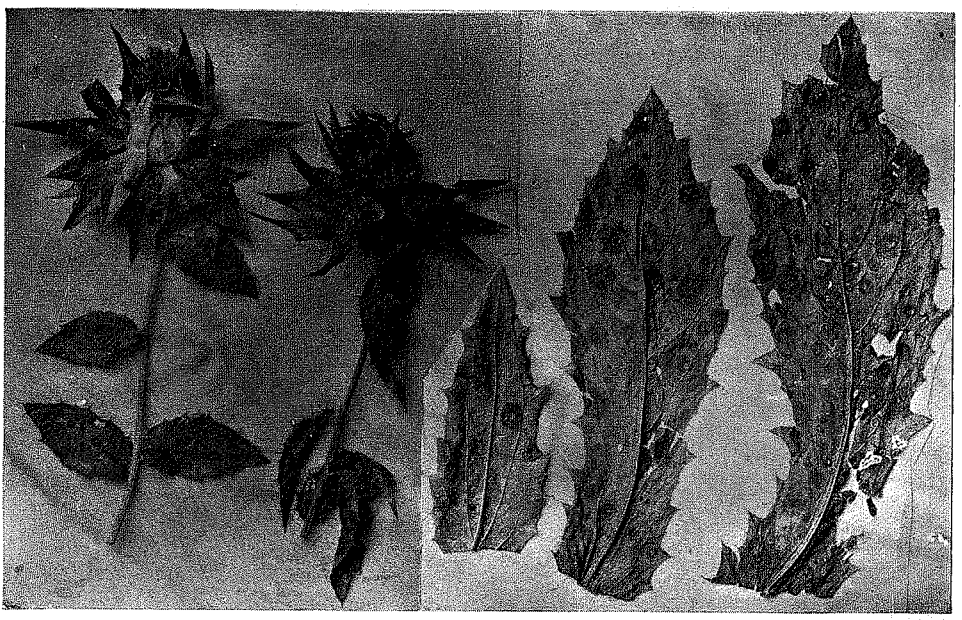

Fig. 1. Flowering twigs and leaves of *Carthamus tinctorius* showing symptoms of the disease. × 1.

Type specimen collected by S. Chowdhury in January, 1936 and deposited in the Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Research Institute, New Delhi.

Alternaria carthami Chowdhury sp. nov.—Hyphis vegetativis septatis, inter et intra-cellularibus, primo subhyalinis, angustis, sparse septatis, aetate fuscis et sæpius septatis, latioribus. Conidiophoris crassis erectis, rigidis, simplicibus, septatis, ad septum tenuiter constrictis, singularis vel fascicularis emergentibus, ex epidermide erumpentibus vel per stomata emergentibus, brunneis, 6–10 m diam. Conidiis pallidebrunneis, pellucidis, muriformibus, ad apicibus conidiophororum singularibus vel catenulatis formantibus, 3–11 cellularis, septis longitudinalis paucis plerumque rostris longis, conidiis 36–99 $\times$12–28 M rostris excluderentibus. Rostro pro parte basili pallidebrunneis, pro parte apic prope hyalino filiformi, 12–84$\times$3–5 M.

Habitat in foliis *Carthamus tinctorius* L. Pusa.

Typus in Herbarium *Cryptogamæ Indiæ Orientalis*, Imperial Agricultural Institute, New Delhi, Leg. S. Chowdhury, January, 1936.

SUMMARY

A leaf-spot disease of *Carthamus tinctorius* was observed at Pusa and its neighbourhood. It has also been observed in other parts of Bihar and in the Central Provinces.

The disease was found to be caused by a species of *Alternaria* hitherto not reported from any part of the world. The morphology and parasitism of the fungus have been studied. It is proposed as a new species for which the name *Alternaria carthami* Chowdhury sp. nov. has been suggested.

The optimum temperature for growth has been found to lie between 25° and 30° C. and the range of optimum hydrogen-ion concentration for growth between 6 and 7.

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