

STUDIES ON THE FOOT ROT AND STEM-ROT OF
HIBISCUS SABDARIFFA VAR. *ALTISSIMA*
HORT. CAUSED BY *PHYTOPHTHORA*
PARASTICA VAR. *SABDARIFFAE* MUK.

BY

NIRMALENDU MUKERJEE

Jute Agricultural Research Institute, I.C.A.R., Barrackpore, West Bengal

(With Plate 1 and 2 Text-figures)

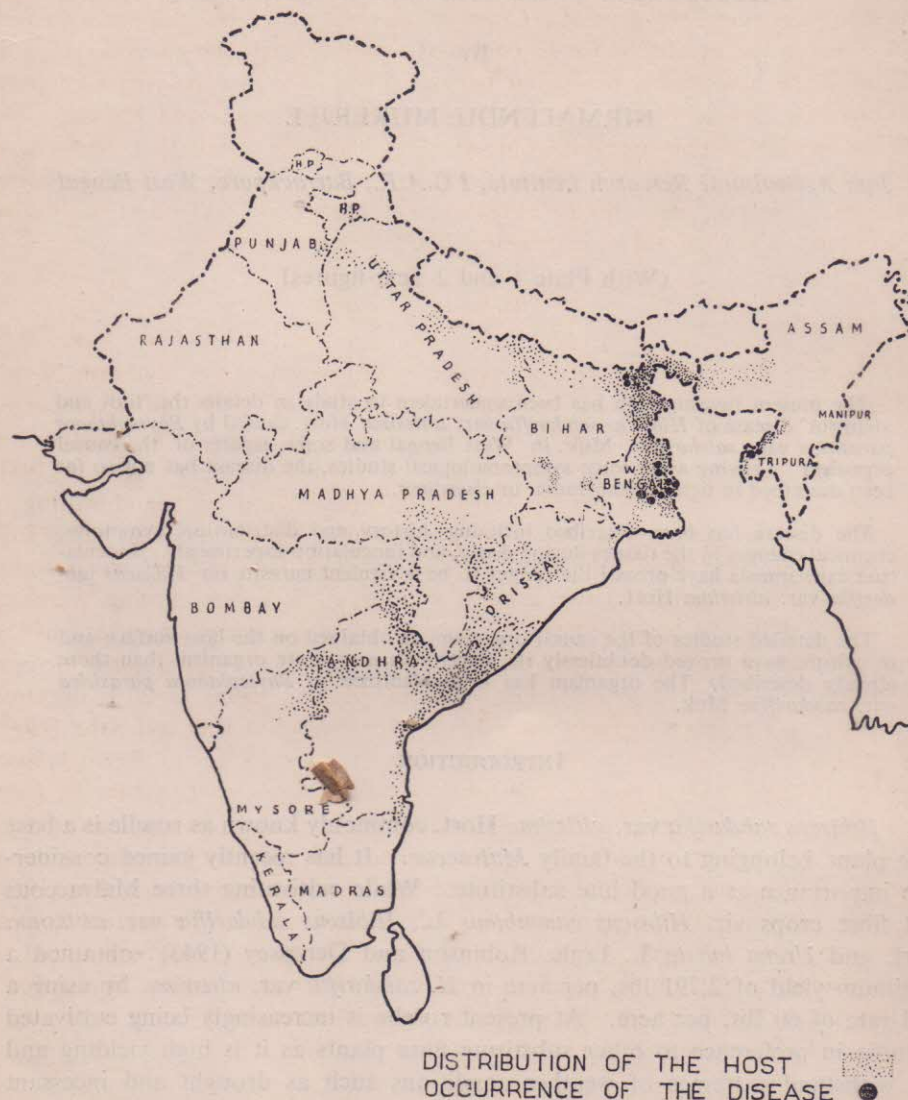
The present investigation has been undertaken to study in details the 'foot and stem-rot' disease of *Hibiscus sabdariffa* var. *altissima* Hort. caused by *Phytophthora parasitica* var. *sabdariffae* Muk. in West Bengal and some aspects of the causal organism. Leaving aside some symptomological studies, the disease has not so far been described in details from India or elsewhere.

The disease has been described including history and distribution, symptoms, chemical changes in the tissues during decay, and inoculation experiments. Inoculation experiments have proved the fungus to be a virulent parasite on *Hibiscus sabdariffa* var. *altissima* Hort.

The detailed studies of the causal organism, as obtained on the host surface and in culture have proved doubtlessly its identity as a separate organism than those already described. The organism has been identified as *Phytophthora parasitica* var. *sabdariffae* Muk.

INTRODUCTION

Hibiscus sabdariffa var. *altissima* Hort. commonly known as roselle is a bast fibre plant belonging to the family *Malvaceae*. It has recently gained considerable importance as a good jute substitute. While cultivating three Malvaceous bast fibre crops viz. *Hibiscus cannabinus* L., *Hibiscus sabdariffa* var. *altissima* Hort. and *Urena lobata* L. Ergle, Robinson and Dempsey (1945) obtained a maximum yield of 2,791 lbs. per acre in *H. sabdariffa* var. *altissima* by using a seed rate of 60 lbs. per acre. At present roselle is increasingly being cultivated in India in preference to other substitute fibre plants as it is high yielding and can withstand extremes of weather conditions such as drought and incessant rain. It can also withstand infestation of weeds and comparatively poor soils. Recently, cultivation of roselle in West Bengal, however, is seriously impeded due to increasing incidence of "foot- and stem-rot" disease. The disease has been noticed in Nilganj, Baduria and neighbouring villages of 24-Parganas, West Bengal during the six years (1955-60) under observation in the months of July and August. It has also been reported from Howrah, Hooghly, and Nadia Districts of West Bengal as well as from Bihar and Tripura (Text-fig. 1).



TEXT-FIG. 1. Map showing the distribution of the host and the occurrence of the disease.

Repeated isolations from diseased plants collected from different places have produced identical luxuriant fungal growth in all cases. After careful observations and detailed study it has been eventually identified as *Phytophthora parasitica* var. *sabdariffae* Muk. Constant association of the fungus with foot and stem-rot disease and by subsequent pathogenicity trials the parasitic relationship with the host has been proved beyond doubt. So far the writer is aware the problem has not been thoroughly investigated in India and abroad. The host being one of the important fibre-yielding plants, it is felt that detailed information regarding the fungus and its association with the host is desirable. With this object in view this investigation has been undertaken and the results of some of the studies are summarised in the following pages.

HISTORY

Gadd (1926) first reported a species of *Phytophthora* (or *Pythium* ?) on *Hibiscus sabdariffa* var. *victor* from Ceylon. Thompson (1929) initially reported the occurrence of *P. parasitica* on *H. sabdariffa* var. *altissima* from Malaya, but later (1933) he mentioned the organism causing wilting of roselle. Hector (1931) reported a species of *Phytophthora* causing brown discolouration and wilting of *H. sabdariffa* var. *altissima* at Dacca (Bengal) Farm. Muller and Van Eek (1939) reported from Java that roselle (*H. sabdariffa*) and Java-jute (*H. cannabinus*) were subject to infection by two groups of fungi in Java, one causing 'foot-rot' and other 'stem and leaf-diseases', the former being the more important of the two. Of the pathogens responsible for 'foot-rot', *Phytophthora parasitica*, is the most troublesome one according to them, causing losses of 20% or more especially on the green stemmed varieties of *H. sabdariffa*. Kar and Saha (1943) reported a disease of roselle from Bengal caused by a species of *Phytophthora*. Lastly, Thung (1947) reported *Phytophthora sabdariffae* on *H. sabdariffa* from Java but no description embodying details of the fungus or its association with the host is, however, available. In recent years (1955-60) a 'foot and stem-rot disease' of *H. sabdariffa* var. *altissimo* Hort. has been noticed by the author (1959) in West Bengal during the months of July and August. The meteorological factors (Appendix-1) and the incidence of disease at N.lganj has been found to be interdependent, and discussed in details in the chapter under 'Discussion'. It may be said that the occurrence of the disease as observed lately is of cyclic nature and the curves are of explosive type.

SYMPTOMS

The infected plants show certain well defined symptoms. Plants above four feet are generally attacked (Pl. 1, figs. 1-3). Firstly, the root-system is infected (Pl. 1, fig. 1). The point of infection may be just below the collar region or much below on the tap and side-roots. At the initial stage of infection an uprooted plant shows moist brownish patches on the tap-root as well as secondary and tertiary roots. Later, the whole root system becomes soft and moist violet-

brown in colour. Simultaneously with this the infection patch spreads upwards to the collar region and above (about 4-6 inches) and becomes chocolate to deep-brown in colour. At this stage or somewhat earlier wilting starts. The leaves become yellowish and droop along with the stem-apex.

Later or simultaneously with root infection, in many plants, lesion is found on the stem. The infection strikes the stem either at the basal region or above spreading upwards and downwards.

When the necrotic patch from the stem reaches ground level it often becomes difficult to determine the seat of infection. In such cases, on uprooting, the roots are usually found healthy indicating the source of infection above ground level. The same plant may have two different seats of infection, one in the root and the other on the stem separated clearly by a green zone in between (Pl. 1 fig. 2).

As the disease proceeds the stem surface cracks at places and exudation of gummy substances takes place through these cracks. In highly advanced stage of the stem-rot, shredding of fibres takes place at the cracked regions and the plants may succumb to wind at these places. Stem-rot in advanced stage causes wilting, the root-system, remaining absolutely free. Cross sections of the stem at the early stage of decay show a moist pale area at the centre with small brownish streaks at the periphery while the stem looks perfectly green outwardly. The lesion at first appears like a streak which spreads longitudinally from the point of infection upwards and downwards, the undersurface remaining white. Later it spreads, laterally and encircles the stem, finally forming a continuous discoloured area around it. This way it proceeds both upwards and downwards. As the disease advances the infected area on the stem surface gradually loses green colour, becomes brownish to chocolate-brown and finally almost black. Simultaneously, the wood surface also changes colour from white to black with brown to deep-brown intergrades (Pl. I, figs. 4-7). At advanced stage, on little pressure the superficial tissues get separated from the surface of the wood and a lot of gumlike substance is found in between them. The surface of this tissue takes a beehive-like appearance and its inner portion shows chocolate red colour with yellowish strands here and there. On further examination, the yellowish strands are found to be completely rotted bast fibres. Even the individual strand can be separated at this stage. The surface of the wood is found to have developed a deep chocolate-brown colour which is present more or less throughout the surface interspersed with pale yellowish strips here and there. However, on the cross-sectional view the deep-chocolate brown colour present on the lateral surface is not found, instead a pale moist yellowish brown central zone with a very faintly brown periphery is seen. At the periphery, rot-pockets are seen which are smaller to start with but become bigger in the advanced stage of the disease. Several rot pockets are found to coalesce with one another. Sometimes pockets are found as narrow hollowed strips as if very minutely sawn in the longitudinal direction. These pockets are found to approach the centre showing the wood getting rotted from the periphery towards the centre. At the advance stage of the disease the wood becomes brittle and breaks on little pressure. In the highly advanced stage dissolution of cells begin at the central por-

tion of the slender stems and finally a bore is formed which follows the advancing zone of the disease on both the sides, i.e., upwards and downwards.

MATERIALS FOR STUDY

The materials for study were collected during the months of July, August and September, 1956-58 at different stages of infection of the host plant *Hibiscus sabdariffa* var. *altissima* Hort. from Nilganj, Baduria (24-Parganas) and several other villages of Howrah, Hooghly and Nadia districts of West Bengal. The soil of these areas was found to be of sandy-loam to loamy in nature. Whenever, materials were collected care was taken to uproot the plant keeping the root-system intact as far as practicable. The temperature and humidity during these months varied between 25°-32°C. and 77-95% respectively. Plants to be studied were carefully selected and infected stems and roots at different stages were cut into small pieces in order to examine microscopically the nature of internal decay. Small, cylindrical sections from the selected portions of the same and different materials were brought to the laboratory examined and studied while fresh. It was also observed that the materials remained workable for 2-3 days if they were kept inside desiccators containing distilled water. But after that, growth of other secondary organisms took place, mainly *Fusaria* making correct study difficult. The cross-section ends as well as the sides showed various stages of discolouration and decay. Microscopic examinations of the rotted areas showed abundant hyaline, coenocytic as well as septate hyphae within the tissues of the host. Chlamydospores and sporangia were found on the surface of the diseased materials.

DECAY OF *HIBISCUS SABDARIFFA* VAR. *ALTISSIMA* HORT.

In search of the mycelium in diseased plants for the purpose of studying its nature and distribution, infected stem and root in all stages of decay were sectioned both free-hand and by a microtome. Free-hand-sections on being suitably stained were generally found to be sufficiently thin to detect the presence of fungal hyphae within the tissue elements. For photomicrography, as well as for studies in detail, microtome sections were better. In most cases, radial, tangential and transverse sections of sound and diseased areas (50-70 μ) were cut without any special treatment, from freshly collected materials with a view to avoid drying and consequent shrivelling of the fungal hyphae. When sections were cut from somewhat dried materials they were given preliminary softening treatment by keeping for 2-3 days in equal parts of methylated spirit and glycerine or simply placing in a vessel of gently boiling water until they became water-logged and sank. In latter case the material was not allowed to stand long enough in water instead sections were cut immediately from the surfaces that had been kept wet

all the time while cutting sections. Such sections, however, were not very satisfactory because in the dried material, the hyphae became somewhat shrivelled rendering microscopic observations difficult. On the otherhand, section from fresh materials were found to be better and the mycelium in the tissues was suitable for study. In most cases radial longitudinal sections showed very clearly the presence of hyphae in all the tissues which were only traccable in the cross-sections. In order to study hyphae in detail within the tissue elements, differential staining by Gentian-Violet and Bismark Brown (Hubert, 1922) and also Saf-ranine and Picro-aniline blue (Cartwright, 1929) were tried.

Of the two, the latter was found to be more satisfactory and easy to manipulate. By this method the lignified structures were stained red and the mycelium blue.

In Stem

In an unstained transection at the initial stage of infection, the epidermis, hypodermis, phloem-rays, phloem-parenchyma and cambial regions are found to exhibit yellow, deep-yellow, brown and deep-brown colourations. The bast fibres, however, are the first element that turn yellowish to a considerable distance, about 7.6 mm downwards from the point of infection, the phloem-rays remaining as usual. Later the fibres become deep-brown and begin to disintegrate from the point of infection. At this stage, the phloem-rays also become yellowish and finally turn deep-brown. Simultaneously with this, gummy materials appear in the fibres. These are at first found to occur in a scattered way but subsequently several fibres in a series appear to contain gummy materials so that long deep-brown streaks are formed. Gum is also found in phloem-parenchyma, ray-cells and vessles. The ray cells gradually become yellow in colour which extend upto the pith. Later the vessels accompanying the wood-parenchyma also become yellow. At this stage all the wood-elements do not appear uniformly yellow but several yellowish vessels are found scattered here and there in between the yellow ray cells. The pith cells at places also turn yellow indicating the progress of the rot to the centre of the stem. These cells appear to be connected with the discoloured ray cells. This indicates clearly that the rot has reached the centre of the stem through the ray-cells. A few fibre-tracheids become yellow near the cambial zone at the initial stage but most of them become yellow later. The exact chemical nature of the coloured cell-wall substance has not been determined. However, similar colouration has been reported by Chatterjee (1958) who states that "following the initiation of infection, a brown coloured substance was detected in the cells and in intercellular spaces of the cortex. The chemical nature of this substance could not be determined by microchemical tests. It was not definitely established whether this brown substance was produced by the pathogen or whether it was a reaction product of the host in response to attack." According to Gothoskar (1955) the cause of brown colouration is not clear, but its nature suggests that phenols are released into the xylem vessles and that the

phenol oxidase system of the plant aids in their conversion to melanins which are absorbed by the lignified xylem walls. The theory is supported by the fact that on introducing weak solutions of certain phenolic compounds into the vascular system of healthy tomato cuttings, prompt vascular browning takes place indicating that the oxidase enzymes are present and functional. In the host under study browning, however, is not restricted to the vessels only and if the above theory holds good it may be said that all the elements showing browning probably having phenols transformed into melanins and subsequently absorbed by the cell walls.

In a stained preparation, at the initial stage of infection, the mycelium is scanty and hyphae are found in phloem-rays, primary-secondary bast fibres and phloem-parenchyma being more abundant in the phloem-rays (Pl. I, fig. 8).

Inside bast fibres they travel more in longitudinal direction. Lateral penetration from one band of bast fibres to the others through uniseriate or multiseriate phloem-rays is also quite common. Even at the initial stage, some of the phloem ray cells become almost filled with the hyphae and bore holes though small are noticeable. Later, two types of hyphae, wider and narrow are found. The width of the former varies between $3.44-6.19\mu$ while that of the latter $1.03-1.72\mu$ usually. The narrower type is more abundantly present. At this time the medullary ray cells are found infected wherefrom the hyphae penetrate the neighbouring vessels. Through the rays and some of the wood elements the hyphae finally reach the centre of the stem but are not uniformly distributed. As the disease advances, the entire cambial zone becomes almost disintegrated and as a result the tissues with the bast fibres of commercial importance become very loose round the woody region. Sectioning at this stage of infection never keeps the external tissues attached to the wood.

In the advanced stage of the disease the distribution of the mycelium in the decayed wood is fairly uniform and hyphae are found in almost all the wood-elements. Of the wider types some take stain and some do not with picro-aniline blue. These hyphae may or may not be septate, branch closely or at intervals. Cell-wall penetration is best shown by these hyphae. While passing from one cell to other, the hyphal tip becomes constricted near the bore-hole on the cell-wall and after emerging through the latter assumes the original size. The process goes on like this. The diameter of this type of hyphae usually varies between $3.44-6.19\mu$. The narrow type is more than the wider and forms a network of hyphae along with the wider types inside the wood-elements. They are usually branched, coenocytic and mostly take stain with picro-aniline blue.

Of the elements the ray-cells are found to be mostly infected (Pl. I, fig. 11) and then come fibre-tracheids and vessels. Many of the ray cells and vessels here and there are found to be occluded with hyphae. Bore-holes in the advanced stage of the disease are found to be abundant and usually vary between $2.7-3.44 (5.1)\mu$ in diameter.

Sometimes two or three fuse to form a bigger borehole with somewhat irregular outline. Occasionally, chlamydospores ($3.44-4.12\mu$ in diameter) are

also found inside the wood elements but they are much smaller than those found on host surface or in cultural media.

In Root

In unstained preparations phloem-rays, phloem-parenchyma and cambial regions appear light brown to deep-brown in colour. The cell-walls of the bast fibres also turn yellowish brown. Majority of the coloured cells show copious deposition of granular gummy substances.

The infection travels to pith through the ray cells mainly. It has been noticed, however, that sometimes from the pith again the infection travels towards the periphery through uninfected tissue-elements. Lateral spreading of the rot also takes place from the infected cells and ultimately the entire root becomes brown to deep-brown in colour.

In a stained preparation both wider and narrow types of hyphae are present. They are both intercellular and intracellular but more abundant within the cells. The narrow type is predominantly present in all the wood-elements. Aggregation of hyphae in the ray cells and fibre-tracheids is more conspicuous though they are also present in the vessels and wood parenchyma. The ray cells almost become clogged with hyphae everywhere from the periphery to the centre. Hyphae spread longitudinally, transversely and laterally but they prefer to spread more in the longitudinal direction. Lateral spreading from ray cells to fibre-tracheids and vessels are also found. Near the centre of the root, vessels uniformly show presence of the hyphae (Pl. I, fig. 10) and get clogged with the same. In highly advanced stage dissolution of the ray-cells and fibre-tracheids takes place which may proceed to such an extent that an entire ray becomes a passage with no cell-wall (Pl. I, fig. 10). Vessels also show dissolution of their walls but these are few and far between.

MICROCHEMICAL STUDIES

Microchemical tests for the detection of lignin and cellulose in sound and partially decayed tissues were carried out. This was done in the usual way by staining the section with Phloroglucin-HCl₆ for lignin and Chlor-zinc-iodine for cellulose. The combination stain, Gentian violet and Bismarck brown which differentiates lignified (violet staining) and unlignified (brown staining) structures and supersedes the old Safranin and Haematoxylin combination, had also been tried. Other micro-chemical stains were also employed most of which agreed closely with the changes indicated by the use of above mentioned stains and will, therefore, not be referred again. Although Harlow (1928) has shown, that these micro-chemical stains do not always give positive proof of the various chemical changes that take place during the process of decay yet the above mentioned stains are still in use in such work and the results obtained so far are tabulated in the following pages.

Table 1. Results of staining with Gentian violet and Bismarck brown for lignin and cellulose in normal and diseased stems of *H. sabdariffa* var. *altissima* Hort.

Elements	LIGNIN	
	Normal	Diseased
Bast fibres	Primary wall of primary and secondary fibres deep-violet to violet, secondary wall of primary and secondary fibres violet to moderately violet.	The tissue containing the bast fibres, phloem-parenchyma, phloem rays and cambium do not remain attached to the section.
Phloem parenchyma	No reaction	
Phloem rays	"	
Cambium	"	
Fibre-tracheids	Primary wall deep-violet, secondary wall violet.	Primary wall violet, secondary wall faintly violet.
Vessels	"	
Wood-parenchyma	Primary wall deep-violet, secondary wall violet.	Primary wall deep-violet, secondary wall violet.
Ray cells	"	Primary wall violet, secondary wall faintly violet.
CELLULOSE		
Bast fibres	No reaction	The tissues containing the bast fibres, phloem parenchyma, phloem rays and cambium do not remain attached to the section.
Phloem parenchyma	Secondary wall brown to deep brown.	
Phloem rays	"	
Cambium	"	
Fibre-tracheids	Secondary wall brown to moderately brown in parts.	Secondary wall deep-brown in parts.
Vessels	Secondary wall brown to deep-brown in parts.	Secondary wall brown in parts.
Wood-parenchyma	"	"
Ray cells	"	"

From table 1 it is observed that the normal stem lamellae (primary walls) of all the wood-elements and ray cells take up deep-violet to violet stains. The secondary walls in part, however, are generally violet in all the wood-elements but the intensity of reaction is rather less. This suggests that lignin is present both in the middle-lamellae and secondary walls to a considerable extent. The secondary walls of phloem parenchyma, phloem rays, cambium and parts of the secondary walls of wood-elements, viz., fibre-tracheids, vessels and wood parenchyma along with ray cells show deep-brown or brown colouration. In diseased stem the tissues outside the woody region do not remain attached to the sections and as such microchemical changes in the bast fibres, phloem parenchyma, phloem rays and cambium cannot be studied in such sections. Of the wood-elements, however, the middle lamellae retain the violet colour though portions of the secondary walls of fibre-tracheids, vessels and ray cells become faintly violet. No remarkable change in colour reaction of the walls of wood-parenchyma is, however, observed. Due to partial delignification the secondary walls of the fibre-tracheids show cellulose reaction more intensely.

Table 2. *Results of staining for cellulose and lignin in normal stem of H. sabdariffa var. altissima Hort. with Chlor-zinc-iodine and Phloroglucin-HCl, respectively.*

Elements	Chlor-Zinc-Iodine Cellulose	Phloroglucin-HCl Lignin
Bast fibres	Primary wall of primary and secondary fibres deep-yellow, secondary wall of primary and secondary fibres greenish yellow.	Primary wall red in both primary and secondary fibres, secondary wall faintly pink in both primary and secondary fibres.
Phloem-parenchyma	Primary and secondary walls somewhat bluish-green.	No reaction.
Phloem ray	Primary and secondary walls deep-blue.	No reaction.
Cambium	Primary and secondary walls somewhat bluish green.	No reaction.
Fibre-tracheids	Primary wall deep-yellow secondary greenish yellow to deep yellow.	Primary and secondary walls pink.
Vessels	"	Primary wall deep-red, secondary wall pink.
Wood-parenchyma	"	"
Ray cells	"	Primary and secondary walls pink.

From table 2 it is evident that in the normal stem the primary and secondary walls of the phloem rays only take up blue colouration showing presence of cellulose. Phloem parenchyma, phloem ray and cambium, however, show a bluish-green colour. In others, viz., bast fibres, fibre-tracheids, vessels, wood-parenchyma and ray cells a deep yellow to greenish yellow colour reaction is noticed in the secondary wall. In presence of cellulosic materials secondary walls of these elements are to take blue colouration but it being less or negligible, the reaction of iodine from Chlor-zinc-iodine with lignin of these cells become very prominent to give the greenish yellow or deep-yellow colour. With Phloroglucin-HCl, the primary walls of the bast fibres become red and those of vessels and wood-parenchyma deep-red. The secondary walls are generally stained pink or light pink.

Table 4. *Results of staining for cellulose and lignin in diseased stem of H. sabdariffa var. altissima Hort. with Chlor-zinc-iodine and Phloroglucin-HCl, respectively.*

Elements	Chlor-Zinc-Iodine Cellulose	Phloroglucin-HCl Lignin
Bast fibres	The tissue outer to the woody region containing bast fibres, phloem parenchyma, phloem rays and cambium do not remain attached in section.	The tissue outer to the woody region containing bast fibres, phloem parenchyma, phloem rays and cambium do not remain attached to the section.
Fibre-tracheids.	Primary and secondary walls moderately yellow.	Primary wall light pink secondary wall moderately pink.
Vessels	"	Primary wall pink secondary wall moderately pink.
Wood parenchyma	"	"
Ray cells	Primary and secondary walls greenish yellow.	Primary wall light pink, secondary wall yellowish.

With Phloroglucin-HCl, the primary walls of fibre-tracheids and ray cells in the decayed stem become light pink to almost colourless while the secondary walls appear yellowish, but the primary walls of vessels and wood-parenchyma, however, show pink colour while the secondary walls moderately pink. With Chlor-Zinc-Iodine the walls of the fibre tracheids become moderately yellow and ray cells greenish yellow. Both these results suggest gradual delignification and it can, therefore, be said that there is considerable delignification of the lignified walls of the fibre-tracheids and ray-cells along with very little or no draining of cellulosic materials.

Table 4. *Results of staining with Gentian violet and Bismarck brown for lignin and cellulose in normal and diseased root of H. sabdariffa* var. *altissima*. Hort

LIGNIN		
Elements	Healthy-root	Diseased root
Bast fibres	Primary walls of primary and secondary fibres violet to deep violet, secondary wall of primary and secondary fibres moderately violet.	Colour reaction is not distinct as they become chocolate brown with infection.
Phloem-parenchyma	No reaction.	"
Phloem ray	No reaction	Colour reaction is not distinct as they become chocolate brown with infection.
Cambium	No reaction.	
Fibre-tracheids	Primary and secondary walls violet.	Primary and secondary walls faintly violet.
Vessels	Primary wall deep-violet secondary wall violet.	Primary and secondary walls faintly violet.
Wood-parenchyma	"	"
Ray cell	Primary and secondary walls violet.	Primary and secondary walls faintly violet.
CELLULOSE		
Bast fibres	No reaction	Colour reaction is not distinct as the zone containing bast fibres, phloem-parenchyma, phloem rays and cambium becomes chocolate brown with infection.
Phloem-parenchyma	Secondary wall brown to deep-brown.	
Phloem rays	"	
Cambium	"	
Fibre-tracheids	Secondary wall brown in parts.	Secondary wall brown in parts.
Vessels	Secondary wall brown to deep-brown in parts.	"
Wood-parenchyma	"	"
Ray cells	"	"

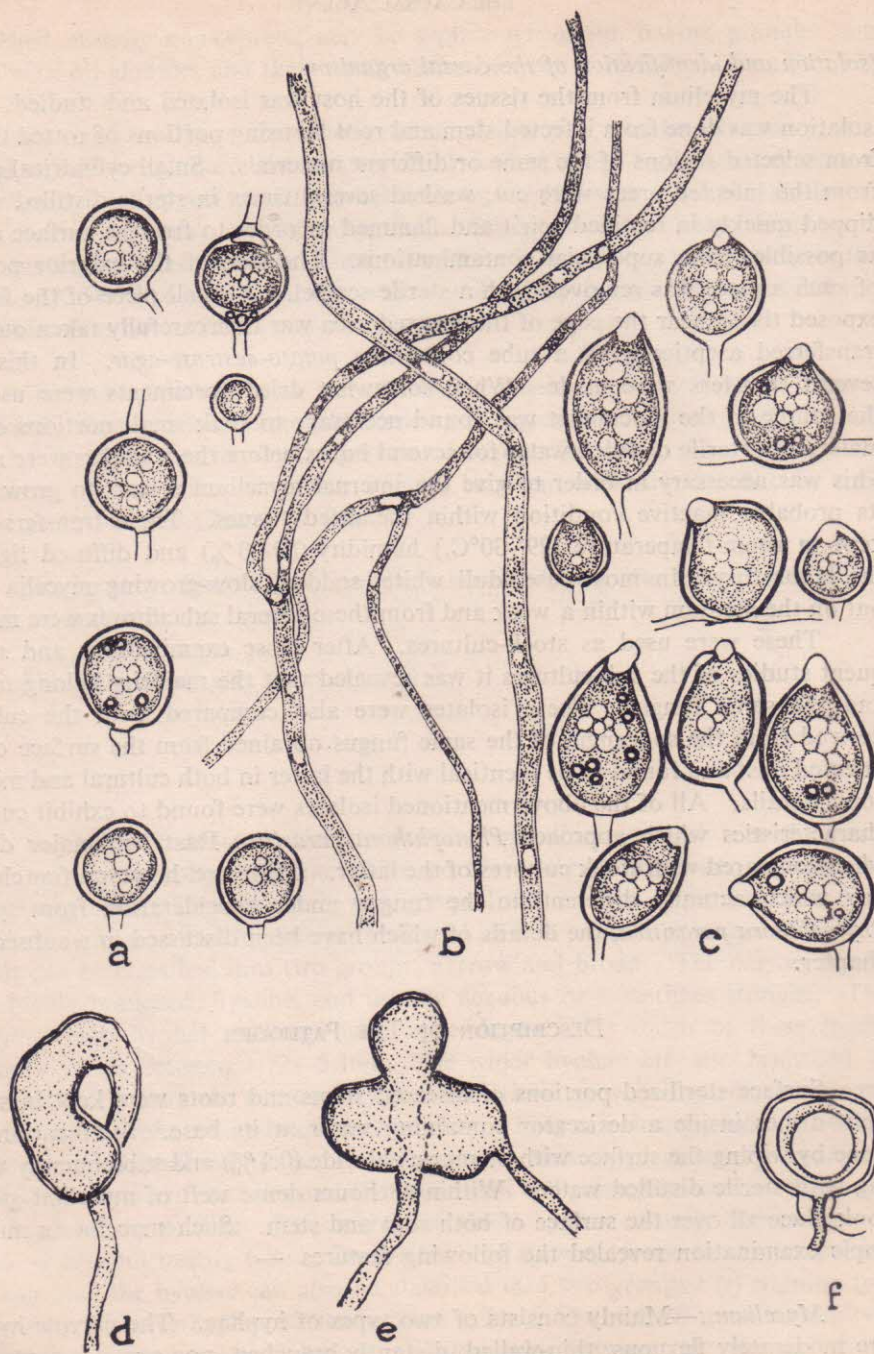
Table 5. *Results of staining for cellulose and lignin in normal root of H. sabdariffa var. altissima Hort. with Chlor-zinc-iodine and Phloroglucin-HCl respectively.*

Elements	Chlor-Zinc-Iodine Cellulose	Phloroglucin-HCl Lignin
Bast fibres	Primary wall of primary and secondary fibres yellow, secondary wall of primary and secondary fibres greenish yellow.	Primary wall red in both primary and secondary fibres, secondary wall faintly pink in both primary and secondary fibres.
Phloem-parenchyma	Primary and secondary walls bluish green.	No reaction.
Phloem ray	Primary and secondary walls somewhat blue.	No reaction.
Cambium	Primary and secondary bluish green	No reaction.
Fibre-tracheids	Primary wall deep yellow secondary yellow to deep yellow.	Primary and secondary walls pink.
Vessels	"	Primary wall deep red, secondary wall pink.
Wood-parenchyma	Primary wall deep yellow secondary yellow to deep yellow.	Primary wall deep red, secondary wall pink.
Ray cells	Primary wall deep yellow secondary yellow to deep yellow.	Primary wall deep red, secondary wall pink.

Table 6. *Results of staining for cellulose and lignin in diseased root of H. sabdariffa var. altissima Hort. with Chlor-zinc-Iodine and Phloroglucin-HCl Respectively.*

Elements	Chlor-Zinc-Iodine Cellulose	Phloroglucin-HCl Lignin
Bast fibres	Colour reaction is not distinct as the tissue containing bast fibres, phloem parenchyma, phloem ray and cambium becomes chocolate-brown with infection.	Same as in Chlor-Zinc-Iodine.
Fibre-tracheids	Primary and secondary walls faintly yellow.	Primary wall very lightly pink, secondary wall yellowish.
Vessels	"	Primary and secondary wall pink.
Wood-parenchyma	"	"
Ray cells	Primary and secondary walls greenish yellow.	Primary and secondary walls light pink.

From the foregoing tables 4, 5 and 6 it appears that microchemical reactions of the walls of the tissue elements in roots both normal and diseased are more or less similar to those of the stem. In roots also gradual delignification takes place in general, but more conspicuous in fibre-tracheids and ray cells.



TEXT FIG. 2. Mycelia, sporangia, chlamydospores and sex-organs of *Phytophthora parasitica* var. *sabdariffae* Muk. (X 446, a—c; X 930 d—f) (Vide Text)

THE CAUSAL AGENT

Isolation and identification of the causal organism

The mycelium from the tissues of the host was isolated and studied. The isolation was done from infected stem and root by using portions of rotted tissues from selected regions of the same or different materials. Small cylindrical pieces from the infected areas were cut, washed several times in sterile distilled water, dipped quickly in rectified spirit and flamed in order to free the surface as far as possible, from superficial contaminations. The side of the exterior portion of such a piece was removed with a sterile scalpel. A small piece of the freshly exposed tissue near the edge of the decayed area was then carefully taken out and transferred aseptically to a tube containing *potato-destrose-agar*. In this way several transfers were made. When somewhat dried specimens were used as the source of the inocula, it was found necessary to soak small portions of the material in sterile distilled water for several hours before the transfers were made. This was necessary in order to give the internal mycelium a start to grow from its probable inactive condition within the dried tissues. These transfers were kept in room-temperature (29°-30°C.) humidity (80-90%) and diffused light of the laboratory. In most case, dull white, sodden, slow-growing mycelia grew out on the medium within a week and from these several subcultures were made.

These were used as stock-cultures. After close examinations and subsequent studies of the sub-cultures it was revealed that the majority belong to one and the same fungus. These isolates were also compared with the cultures derived from the sporangia of the same fungus obtained from the surface of the infected host and found to be identical with the latter in both cultural and microscopic details. All of the above mentioned isolates were found to exhibit cultural characteristics which approach *Phytophthora parasitica* Dast. in major details when compared with stock cultures of the latter. There are, however, few characters which actually differentiate the fungus under consideration from typical *Phytophthora parasitica*, the details of which have been discussed in a subsequent chapter.

DESCRIPTION OF THE PATHOGEN

Surface sterilized portions of diseased stems and roots were kept in sterile Petri-dishes inside a desiccator containing water at its base. Sterilization was done by wiping the surface with mercuric chloride (0.1%) and subsequently washing with sterile distilled water. Within 48 hours dense weft of mycellial growth took place all over the surface of both root and stem. Such mycelia on microscopic examination revealed the following features :—

Mycelium :—Mainly consists of two types of hyphae. The narrow hyphae are moderately flexuous, thin-walled, distantly branched, non-septate, containing granular contents with oil globules and their width varies between 2.75-3.44 μ . The wider type, on the other hand, are mostly straight, thin-walled, distantly

branched, usually non-septate, may be septate when old, having granular contents with oil globules and their width varies between $5.16-6.88\mu$ (TEXT FIG. 2b).

Sporangia :—These are usually terminal but may also be intercalary, one-celled, ovoid or globose, moderately thick-walled, full of granular cytoplasm, and usually one lipped (papillate) but may be rarely bilipped. The papillae are very distinct and may be somewhat flat to highly convex. The dimension of the sporangia varies between $20.64-61.92 \times 17.20-48.16\mu$ but mostly within $24.08-37.84 \times 20.64-30.96\mu$. The width of the sporangiophores varies between $2-3.44\mu$ (TEXT-FIG. 2,c).

Chlamydospore :—These are both terminal, and intercalary, mostly spherical, thick-walled, one-celled and with granular contents. The width of the hyphae bearing chlamydospores varies between $3.44-5.16\mu$. The diameter of the chlamydospores ranges between $6.88-36.12\mu$ but in most cases $15.48-24.08\mu$ (TEXT-FIG. 2,a).

Sex organ :—These have never been observed even after repeated examinations on infected host tissues.

Morphological Studies in vitro on P.D.A.

Mycelium

Initially a sodden moderately growing mycelium is seen. On the fourth day the aerial hyphae become white, cottony cob-webby with an appressed advancing zone. Comparatively young mycelium consists of thin, branched and aseptate hyphae. The hyphae at the initial as well as in later stages of development can be classified into two groups, narrow and broad. The narrow hyphae are highly branched, hyaline, and usually flexuous or sometimes straight. They may develop hyphal swellings with appendages. The width of these hyphae usually varies between $1.72-5.16\mu$. The wider hyphae are also branched but less frequently than the narrower type. Sometimes a swollen structure is formed on the main hypha which bears the branches. They are usually hyaline, straight and possess hyphal swellings. In old cultures, these hyphae become more septate than the narrower ones and the individual cells then become barrel-shaped or irregular in outline. The width of these hyphae usually varies between $5.16-9.63\mu$ but mostly 6.8μ . On the basis of staining properties in lactophenol cotton blue the hyphae can also be classified into two groups : (i) staining type, the width of these hyphae varies between $1.72-9.63\mu$ and (ii) non-staining type, —width ranging between $3.44-6.88\mu$. Intergrades between the two can also be seen. The cytoplasm of both these types of hyphae is finely granular, vacuolated and intermingled with oil globules or other bodies. In older hyphae, the granules become coarser and aggregate into groups forming irregular masses.

Sporangiophores and Sporangia

The sporangia are borne on simple stalks or sporangiophores. According to Blackwell (1949) the sporangiophore may be classified as simple, consisting of a single monochasial sympodium with lax sub-sporangial branching. The new branch initial is developed outside and below the base of the previous sporangium. The width of the sporangiophore varies between $2-3.5\mu$. The sporangia are usually terminal or intercalary but may also be lateral. They are hyaline, one-celled and usually ovoid to pear-shaped. Intercalary ones may, however, be round or ovoid in form. Each sporangium usually has one terminal papilla but some are found to have two. The dimension of the sporangia varies between $27.52-72.24 \times 20.64-29.24\mu$. The cytoplasm is dense and granular in both sporangia and sporangiophores. Abnormal sporangia both terminal and intercalary are also sometimes met with.

Chlamydospores

These are usually spherical, terminal or intercalary the latter being more frequent than the former, thick but smooth walled and somewhat yellow in colour. Intercalary chlamydospores may be distantly separated or may be close to one another in chains. Their diameter usually varies between $20.64-37.84\mu$.

Sex-organs

The sex-organs have been obtained once only from the mycelium during isolation. The number of antheridia and oogonia are, so few that their development could not be studied in early stages. The antheridia are amphigynous (Pl. I, fig. 12) and measure about $34.4 \times 13.76\mu$ in their upper portions (TEXT-FIG. 2, d-e). Only a few oospores within partly collapsed oogonia have been observed. The diameter of the oospores varies between $17.20-27.52\mu$ (TEXT-FIG. 2, f). Various solid media viz., oat-agar, host-decoction-agar, malt-agar, nucleic acid-agar and potato-dextrose-agar have been tried for the production of sex-organs and oospores but without success at different temperatures viz. $20-23^{\circ}\text{C}$, 30°C , and 35°C . However, the author (1962) has observed that a microbial association is necessary for normal production of sex-organs and oospores.

Zoospores

It has been found by the author (1967) that zoospores are formed under optimum temperature varying between $24-27^{\circ}\text{C}$. They do not germinate within half an hour like those of *P. parasitica* but mostly germinate from one hour onwards. Details of zoospore formation and their behaviour have been worked out and will be found in reference cited above.

GERMINATION OF SPORANGIA AND CHLAMYDOSPORES

A. Sporangia

The method by which sporangia germinate is variable. Normally, two main types of germination are encountered and known respectively as direct and indirect. In the former, germination takes place by the formation of one or more germ-tubes while in the latter by the production of zoospores. The zoospores after a period of swarming come to rest and each gives rise to the mycelium. It has also been observed that a sporangium which has just started germinating directly may give rise to zoospores under favourable conditions.

Direct germination :—This is the most common type of sporangial germination met with in abundance on different solid media. The number of germ-tubes, however, is not always constant. They are at first straight and may produce few branches. The germ-tubes usually arise from both the ends of the sporangium but occasionally lateral germ-tubes are also formed. It has been observed that the number of germ-tubes at the papillar end is usually more than those at the proximal end. In the latter, they come out from the point where the sporangium remains attached to the sporangiophore. At the papillar end the germ-tube or tubes arise usually from the side of the papilla or a little below it, but sometimes direct prolongation of the papillar end in the form of a germ-tube is also met with. Sometimes the germ-tubes at the papillar end appear to arise in a whorl or cluster. The abnormal sporangia also germinate by producing germ-tubes.

Indirect germination :—It has already been stated that the sporangia under favourable conditions of temperature produce zoospores. These zoospores on coming out from the sporangia swim about 40-45 minutes under the existing room-temperature (26°C). As they come to rest they do not germinate immediately but usually take about 60 minutes to give rise to new hyphae, though sporadic germination after 40-45 minutes are also observed.

B. Chlamydozoospores

Both terminal and intercalary chlamydozoospores have been found to germinate by putting forth germ-tubes or by the production of zoospores or both by germ-tubes and zoospores. The number of germ-tubes is usually one but in some cases upto four are also found coming out from the periphery of the spore-wall. However, zoospore-formation in the chlamydozoospores may be said to be not so common as in sporangia but that zoospores are formed from both terminal and intercalary chlamydozoospores is unmistakable.

INOCULATION EXPERIMENTS

In order to prove the pathogenicity of the fungus against the host several experiments were done as follows :—

Preparation of the inoculum :—A soil medium was prepared by mixing 190 gms. of well-meshed virgin soil, 10 gms., of maize-meal and 90 c.cm. of distilled water as recommended by Prasad and Padwick (1939) with slight modification. The above mixture was taken in flasks and sterilized by autoclaving at 20 lbs. pressure for one hour. The inocula of the fungus from a 7 days-old *potato-dextrose-agar* culture were placed in the sterilized flasks and allowed to grow for a month. After the growth, the medium was taken out from each flask and the fungus was well mixed with the medium by means of a sterile bamboo chip. This served as the inoculum for immediate use.

Inoculation :—Twenty four pots (10" in diameter) were first cleaned with water and sterilized by formaldehyde vapour for twenty-four hours by keeping them inverted over crucibles containing commercial formalin. After the treatment the pots were kept in vertical position with their mouth upwards to allow formaline to evaporate. A set of twelve such pots was then filled with sterilized soil (about 2/3) and the other set of twelve pots with unsterilised soil in the same way. On soil-surface, the inoculum was sprinkled in eight pots of each set and four were kept as control. Eight healthy seeds in each of these sixteen pots were placed at suitable distances over the inoculum and covered with a thin layer of sterilized and unsterilized soil as the case may be. In the eight control pots of two sets, eight seeds were also sown in each pot and covered with sterilized and unsterilized soil in a similar way. The two sets of pots were then kept separately near window. Arrangement for light at night was also done. Watering was done regularly. The results for of the experiment are given in the following Tables 7 and 8.

The seedlings at the cotyledonary stage within 24-48 hours of germination became infected. The first visible symptom was shown by the cotyledons which turned yellow, then yellowish brown and finally moist black. Subsequent examination showed that primary root in all the cases was found infected before the initiation of secondary roots. The infection in the root was usually found to travel first from up downwards though lesions could also sometimes be found throughout the primary root. Subsequently it proceeded into the aerial parts. An infected root looked moist yellowish brown to blackish brown in colour. Re-isolations were made from different regions of the seedling, viz., root, hypocotyl and cotyledons. In all cases *Phytophthora parasitica* var. *sabdariffae* was always reisolated.

On microscopic examinations of the infected parts coenocytic hyphae were found to be present throughout. They spread longitudinally as well as laterally but preferred to travel lengthwise. Outlines of the hyphae were smooth or wavy with swellings here and there and could be straight or highly flexuous, filled with cytoplasmic contents and oil bodies. Branching was not regular but at places it was conspicuous though unbranched hyphae of considerable length were common. The greatest length that could be measured of a hyphae travelling

Table 8. Results of inoculation experiment in pots containing unsterilized soil.

Date of inoculation and sowing	No. of seeds per pot	No. of seeds germinated per pot	No. of plants infected per pot	Part of the plant initially infected	Serial no of pot	No. of hrs. when symptoms were first observed after germination	Anatomical observations	Organism reisolated
	8	8	6		*H.S. ₁ (N.S.)			
	8	5	5		HS. ₂ (N.S.)			
	8	5	3		H.S. ₃ (N.S.)			
	8	6	6	Root	H.S. ₄ (N.S.)	24-48 hrs.	The tissue elements of the diseased portions of the seedlings were found infected with coenocytic hyphae. In some cases sporangia of the fungus were also seen.	<i>Phytophthora parasitica</i> var. <i>sabdariffae</i> .
13.5.58	8	7	6		H.S. ₅ (N.S.)			
	8	7	5		H.S. ₆ (N.S.)			
	8	6	3		H.S. ₇ (N.S.)			
	8	4	4		H.S. ₈ (N.S.)			
Control	8	8	—		*H.S. (N.S.) C ₁			
	8	8	—		H.S. (N.S.) C ₂			
	8	8	—	No infection	H.S. (N.S.) C ₃			
	8	8	—		H.S. (N.S.) C ₄			No organism isolated

*H.S. (N.S.) = *Hibiscus sabdariffa* var. *altissima* in non-sterilized soil

H.S. (N.S.) = " " " "

C₁C₄

longitudinally without branching was 190.57μ . Sometimes branching took place very closely resulting in a cluster of hyphae or in otherwards might look like scorpioid cymes. The width of the hyphae varied usually between $3.44-6.88$ (8.63) μ . Sometimes sporangia and chlamydospores were also found in the teased materials from diseased portions of the seedlings (Pl. I, fig. 9).

Soil inoculation under field conditions.

Soil preparations and method of inoculation were just the same as in experiment no. 1. After inoculation the pots were kept in the experimental garden outside the laboratory under the prevailing atmospheric conditions. Watering was done regularly. The results will be evident from the following Table Nos. 9 and 10.

The pots were first inoculated on 23rd May, 1958. No symptoms were noticeable upto 20th June, 1958 and all the pots excluding the control were re-inoculated on the same day. While applying inocula for second time, surface soil from base of the plants were carefully removed to expose the root-region as far as practicable. The gap which was caused by removal of surface soil was refilled with soil-inoculum. The plants were kept under observation. No symptom developed within one month. Inoculum for the third time was applied in a similar way on 25th July '58 and the plants kept under observation. After the third application of inocula, incipient infections were noticed in several plants in both the sets within 26-32 days. Between 38-40 days, 9 plants (4 in sterilized set and 5 in unsterilized set) showed characteristic symptoms of *Phytophthora* infections as already stated.

Stem-inoculation by wounding

Ten pots, each having three 63 days-old healthy host plants were selected for the experiment. Of these, nine plants in three pots were kept as control.

In each plant two incisions were made, one in the middle of the stem and the other a little above its basal region. The procedure adopted was that of Banerjee (1956A) which involved in making an incision resembling two upper arms of a triangle on the surface of the stems. Such wounded portions were inoculated by transferring bits of 12-days-old actively growing mycelium from tissue cultures of *Phytophthora parasitica* on *potato-destrose-agar* by carefully lifting the thin flap of peripheral tissue. Prior to inoculation, the surface of the stem was wiped with rectified spirit and washed subsequently with sterile distilled water in order to free the surface, as far as possible, from superficial contaminations. Each wound was then covered with a piece of moist, absorbent, sterile cotton wool and a piece of cellophane paper over it, in order to prevent quick drying out and firmly bound with a string. The string and the cotton wool were removed after 72 hours. The control plants were also treated in the same way but the inocula were without the fungus.

Table 9. Results of inoculations in pots containing sterilized soil under field conditions.

Date of sowing	Date of inoculation	No. of seeds per pot	No. of seeds germinated per pot	No. of infected plants	Seat of initial infection	Serial No. of pots	No. of days when symptoms first observed	Anatomical observations	Organism reisolated
		8	6	—	—	*H.S. ₁ (S.S.)		Coenocytic hyphae both wide and narrow were found in all the tissue elements of root and infected portions of stem. A type of wide septate hyphae was also noticed.	<i>Phytophthora parasitica</i> var. <i>sabdariffae</i> .
		8	5	—	—	H.S. ₂ (S.S.)			
	23.5.68	8	5	—	—	H.S. ₃ (S.S.)			
	20.6.58	8	8	1	Root	H.S. ₄ (S.S.)			
		8	8	2	Root	H.S. ₅ (S.S.)	100 to 102 days		
23.5.58	25.7.58	8	5	—	—	H.S. ₆ (S.S.)			2
		8	8	1	Root	H.S. ₇ (S.S.)			
		8	5	—	—	H.S. ₈ (S.S.)			
		8	7	—	—	H.S. (S.S.) C ₁			
		8	8	—	—	H.S. (S.S.) C ₂			
Control		8	5	—	No infection	H.S. (S.S.) C ₃			No organism isolated
		8	7	—	—	H.S. (S.S.) C ₄			

*H.S. (S.S.) = *Hibiscus sabdariffa* var. *altissima* in sterilized soil
H.S. (S.S.)
C₁—C₄ (Control pots)

Table 10. Results of inoculations in pots containing unsterilized soil under field conditions.

Date of sowing	Date of inoculation	No. of seeds per pot	No. of seeds germinated per pot	No. of infected plants	Seat of initial infection	Serial No. of pots	No. of days when symptoms first observed	Anatomical observations	Organism reisolated
		8	8	—	—	*H.S. ₁ (N.S.)			
		8	8	—	—	*H.S. ₂ (N.S.)			
		8	7	—	—	H.S. ₃ (N.S.)			
		8	8	3	Root	H.S. ₄ (N.S.)		Coenocytic hyphae both wide and narrow found in all the tissue elements of diseased portions. Septate hyphae were also noticed.	
		8	6	—	—	H.S. ₅ (N.S.) 96 days to 100 days			
23.5.58	23.5.58	8	8	1	Root	H.S. ₆ (N.S.)			<i>Phytophthora parasitica</i> var. <i>sabdariffae</i> .
	20.6.58	8	8	1	Root	H.S. ₇ (N.S.)			
	25.7.58	8	7	—	—	H.S. ₈ (N.S.)			
Control		8	3	—	—	*H.S. (N.S.) C ₁	—	—	
		8	7	—	—	H.S. (N.S.) C ₂	—	—	
		8	7	—	—	H.S. (N.S.) C ₃	—	—	
		8	6	—	—	H.S. (N.S.) C ₄	—	—	No organism isolated.

*H.S. (N.S.) = *Hibiscus sabdariffa* var. *altissima* in non-sterilized soil
H.S. (N.S.) = " " " " " " (Control pots)
C₁—C₄

The results of the experiment are shown in table 11.

Table 11. *Results of stem-inoculation by wounding.*

No. of pots	No. of plants in each pot	Age of the plants in days	Date of inoculations	No. of inoculations	Age of the fungus in culture (days)	Bandage opened on	Remarks
7	3	63	25.7.58	2	12	28.7.58	All plants infected
3	3	63	25.7.58	2	—	28.7.58	None infected

On removing the bandage, lesions were noticed in all plants at the points of inoculation, leaving aside the plants which were kept as controls. The plants were then kept exposed to natural atmospheric conditions. The lesion at first appeared as a streak but subsequently it grew lengthwise and laterally. Within 5-6 days the stem at the point of infection was completely encircled by the lesion. It then proceeded upwards and downwards encircling the stem. Wilting was eventually noticeable within a fortnight from the date of inoculation. However, all the infected plants did not show the same degree of infection. Those in which infection advanced more rapidly showed complete wilting within 18-19 days. During this time the lesion was found to have covered an area of about 14.3 cm. above and 10.3 cm. below the point of infection at the middle region of the stem. Near the basal region, however, the lesion was found to cover an area of about 4.5 cm. above and 16.5 cm. below the point of infection. The collar as well as the whole root-system became infected finally. The roots turned reddish and soft. On microscopic examinations numerous hyphae were seen within the infected tissues of stem and root. This showed that the fungus could travel downwards from the stem into the root system. It was generally noticed that the progress of infection was more in the upper region of the stem than that at the basal portion. On reisolutions from the diseased areas *P. parasitica* var. *sabdariffae* was obtained.

Stem inoculation without wounding

A. By contact

Several potted plants which were infected by wounding and in which necrotic patches advanced to some extent were brought very close to the pots containing healthy plants. The stem of a healthy plant by the side of a diseased plant was tied with the diseased portion by a thread ensuring no injury to the healthy stem. These plants were kept under prevailing atmospheric conditions. Within 4-5 days infection patches developed on all the healthy plants in contact with the diseased stems. The results will be evident from the table 12.

Table 12. Results of stem infection by contact.

Date of contact	No. of healthy plants	No. of plants taking up infection due to contact	No. of days within which infection became visible	Remarks
30.7.58	18	15	4-5	Healthy plants take up infection when they come in contact with the diseased portions of infected plants under favourable atmospheric conditions without wounding.

B. By sporangial spray

Ten plants about 1-month-old and 1.5' in height were brought to the laboratory from the field. Soil attached to root-system was carefully removed under tap as far as practicable. The plants were showing only physiological wilting due to disturbed water balance in the meantime. Immediately after washing, the plants were fixed in conical flasks, the root-system being submerged in 200 c.cm. of normal Hoagland's solution in each. One plant was placed in each flask and the mouth was sealed with paraffin. Within 4 hours, the plants recovered fully from wilting, became upright and appeared perfectly normal.

Eight such plants were sprayed with a suspension of sporangia and chlamydospores of the pathogen in sterile distilled water. The suspension was made from a fresh culture of the fungus on *potato-dextrose-agar*. Two plants were kept as control which were sprayed with simple sterile distilled water. After spraying, all the plants were kept under moist bell-jars near window. The control plants were, however, kept separately. These were opened at intervals of twelve hours and the plants sprayed with sterile distilled water each time. None of the plants showed any symptom of infection even after one and a half month. What factors prevented the plants in taking up infection could not be stated with certainty.

It may, however, be assumed that excessive moisture in the bell-jars alone or in collaboration with some other unknown factors acted adversely either upon the germination of the sporangia and chlamydospores or on those which had already germinated.

Spraying of sporangial suspension was also done in the potted plants kept in the field on the same day as above. There also plants showed no symptom within a month. This may be due to unfavourable air-temperature preventing the germination of sporangia and chlamydospores.

C. By placing agar-culture in contact with the stem

Ten plants as stated above were arranged in conical flasks. Eight plants were inoculated at the apex on the stem, and 2-3 axils of leaves near the apical

region of the stem without imparting injury to the plants. The inocula were taken from 12-days-old culture of the fungus on *potato-dextrose-agar*. The inoculated plants were kept in moist bell-jar, and placed very near to a window where light was available throughout the day. During night light was supplied by a fluorescent tube. The two flasks, used as control, were kept under the same conditions separately.

Within 48 hours, infection patches were visible at the apices and axils of all the plants. The axillary buds with leaf primordia dried up and became chocolate brown. The stem adjacent to the axil developed a brownish yellow patch. The apex also became chocolate brown in colour to start with and finally dried up killing the growing tip. In some cases, infection travelled to the lamina through petiole. On re-isolations *P. parasitica* var. *sabdariffae* was obtained. Control plants remained perfectly healthy. The results are given in table 13.

Table 13. Results of placing agar-culture of the pathogen on stem without wounding.

Date of inoculation	No. of plants	No. of plants taking infection	Date of which symptoms first observed	Remarks
7.7.58	8	8	9.7.58	Infection in all cases.
	2	None	9.7.58	No infection

DISCUSSION

The present investigation on the biology of the causal organism *Phytophthora parasitica* var. *sabdariffae* Muk. and its parasitism on *Hibiscus sabdariffa* var. *altissima* Hort. invites discussion in a general way the factors influencing the relationship of the fungus with the host in nature, identification of the organism and some other salient features.

It has been found that the number of diseased plants and disease percentage considerably increase between the last week of August and the third week of September and fall sharply between the fourth week of September and the second week of October in the experimental field at Nilgunj Barrackpore, West Bengal. It has further been observed that 'foot-rot' decreases after the third week of September but stem-rot does not. The data on being plotted different curves have been obtained. In finding out the reasons for this different behaviour the meteorological data (Appendix 1) have been found to be helpful. When 'foot-rot' and 'stem-rot' curves are drawn along with the meteorological data it has been noticed that 'foot-rot' can be correlated with the soil-temperature and rainfall and 'stem-rot' with air-temperature. It has been observed that between the date of sowing and the first record taken, though the rainfall is 14.64" there is less incidence of 'foot-rot'. This can be explained by the fact that during the period rain is so distributed that it can not bring down the soil temperature (15 cm. depth) which remains at 89°F. (=31°C.). In

between first, second and third records, though the total amount of rainfall is slightly more, i.e., 15.83. "yet the incidence of 'foot-rot' is more due to the fact that the soil temperature comes down to 84.8-85°F. (=29.5°C.). In between third and fourth records the rainfall becomes less, i.e. 3.45" and the temperature of the soil also goes up to 87°F. (=30.5°C.) resulting lesser 'foot-rot'. This corroborates that rainfall and soil temperature are correlated with foot-rot.

Soil temperature, however, directly influences the incidence of 'foot-rot' while rainfall does it indirectly. Its influence on soil-temperature is such that if rainfall is wide-apart and cannot bring down the temperature of the soil, the correlation ('foot-rot' and rainfall) will not stand even if the total amount rainfall is quite high during a particular period. From cultural studies also it has been found that above 30°C. the fungus has a tendency to grow less and at and above 35°C. it generally refuses to grow. It can, therefore, be inferred that as the temperature comes down in the soil, the fungus gets a better chance to grow and infect plants. In other words, lower soil-temperature helps to increase 'foot-rot'. Similarly, in case of 'stem-rot' it will be noticed that air-temperature (26°-32°C) is very favourable for the aerial spreading of the fungus during August to the second week of October. The cultural studies have also shown that such a high temperature as 30°C. though produces copious aerial growth of the fungus, yet it is the lower temperature (21°-23°C) that helps in the formation of asexual reproductive bodies though in host-decoction agar they are produced even at 30°C. but the number increases considerably at a lower temperature (21°-23°C). This possibly points to the fact that in nature the fungus can grow on the host even at 30°C. but the performance is definitely better at a somewhat lower temperature. Hence there is every possibility that the fungus gets an upper hand in spreading the disease by sporangia and chlamydospores at somewhat lower range of temperature than at high temperatures. Further, when the temperature comes down to 26°C. there is every possibility of zoospores helping in spreading the disease further. In this way air-temperature can be correlated with aerial infection. It can, therefore, be inferred that during the moonsoon (particularly during August-September at Nilgunj) when the temperature of the soil and the air comes down, the fungus gets a better opportunity to grow and reproduce vigorously. Possibly, this is the most important factor in increasing both 'foot-rot' and 'stem-rot'.

Zoospores are produced by sporangia and chlamydospores under optimal conditions, but more by the former. In case of sporangia, the zoospores come out through the aperture at the papillar end but in case of chlamydospores the zoospores usually cannot come out as there is no opening like sporangia. Such zoospores inside chlamydospores germinate normally, the germ-tubes, however, make their way out penetrating the wall. Under unfavourable temperature conditions rounding of cytoplasm prior to zoospore-formation takes place in both chlamydospores and sporangia. Instead of true zoospores being formed, the cytoplasm gives rise to several globular bodies which are much smaller than normal zoospores. They do not show movement nor they germinate producing

new hyphae. Under these conditions, sometimes, the wall of the chlamydospore has been found to rupture making an exit for them. In the sporangia, however, they come out through the papillar end. Some chlamydospores germinate directly even when zoospores are already formed inside as some sporangia. Hence in this fungus it is found that both sporangia and chlamydospores produce zoospores, and can germinate by producing hyphae directly or an individual may produce both hyphae and zoospores. Between them some morphological features are different such as the presence of papilla in sporangia and the difference in the shape of the two (though sometimes the sporangia are almost spherical resembling a chlamydospore but for the presence of very small papillae they are not mistaken morphologically). Further, chlamydospores have been found to occur in chains like intercalary sporangia. They also arise from the same hyphae that produce sporangia. All these features of chlamydospores, however, enable the writer to say that they do not act only as resting spores but have potential to function like sporangia.

In *Phytophthora parasitica* Dast., the author (1913) mentions that germination of sporangia takes place in three different ways. Firstly, by giving rise to mycelium directly. Secondly, by the formation of zoospore which germinate readily within half an hour. Lastly, by forming one or more short germ-tubes which give rise to secondary sporangia. These secondary sporangia may either form zoospores or tertiary sporangia at the end of short germ-tubes. It is not unusual to find three or four generations of sporangia linked together by short germ-tubes. This last type of germination is not found in the fungus under consideration. The zoospores in this case also do not germinate within half an hour but preferably from one hour onwards from the time of cessation of their movements. Venkatakrishnaiya (1950) while describing *Phytophthora parasitica* on different media states that it makes a dense, white, fluffy aerial growth on oat and onion agars but not on potato-dextrose-agar or French-bean-agar. Dastur (1913) described his fungus on potato-juice-agar (150-10-500) as "growth in this medium was not localised, it was mostly in the substance of the medium and there was a very sparse aerial growth sporangia were formed freely both in the aerial and submerged mycelium but none of these were found to produce zoospores. They germinated conidially without falling off from their conidiophores. They were abnormal both in size and shape and contained a lot of oil globules. "The fungus under consideration, however, has got luxuriant growth of white, cottony-cobwebby aerial mycelium in the potato-dextrose-agar at (30°C. and 21-23°C.) and produces normal sporangia and zoospores. Further, in cultural studies (to be published elsewhere) the fungus in question behave almost like *Phytophthora parasitica* or varieties of the same according to Leonian's (1925) schedule in most of the reactions. But it has not shown characters fitting exclusively with *Phytophthora parasitica* Dast. nor any of its varieties. Unfamiliar reactions have been shown with the amino-acids in general and magnesium nitrate. The fungus also refuses to form sex-organs and oospores unlike *Phytophthora parasitica* Dast. on oatmeal. All these features together point to the fact that the fungus under consideration is a new varie

of *P. parasitica* and has therefore been called as *Phytophthora parasitica* var. *sabdariffae* Muk.

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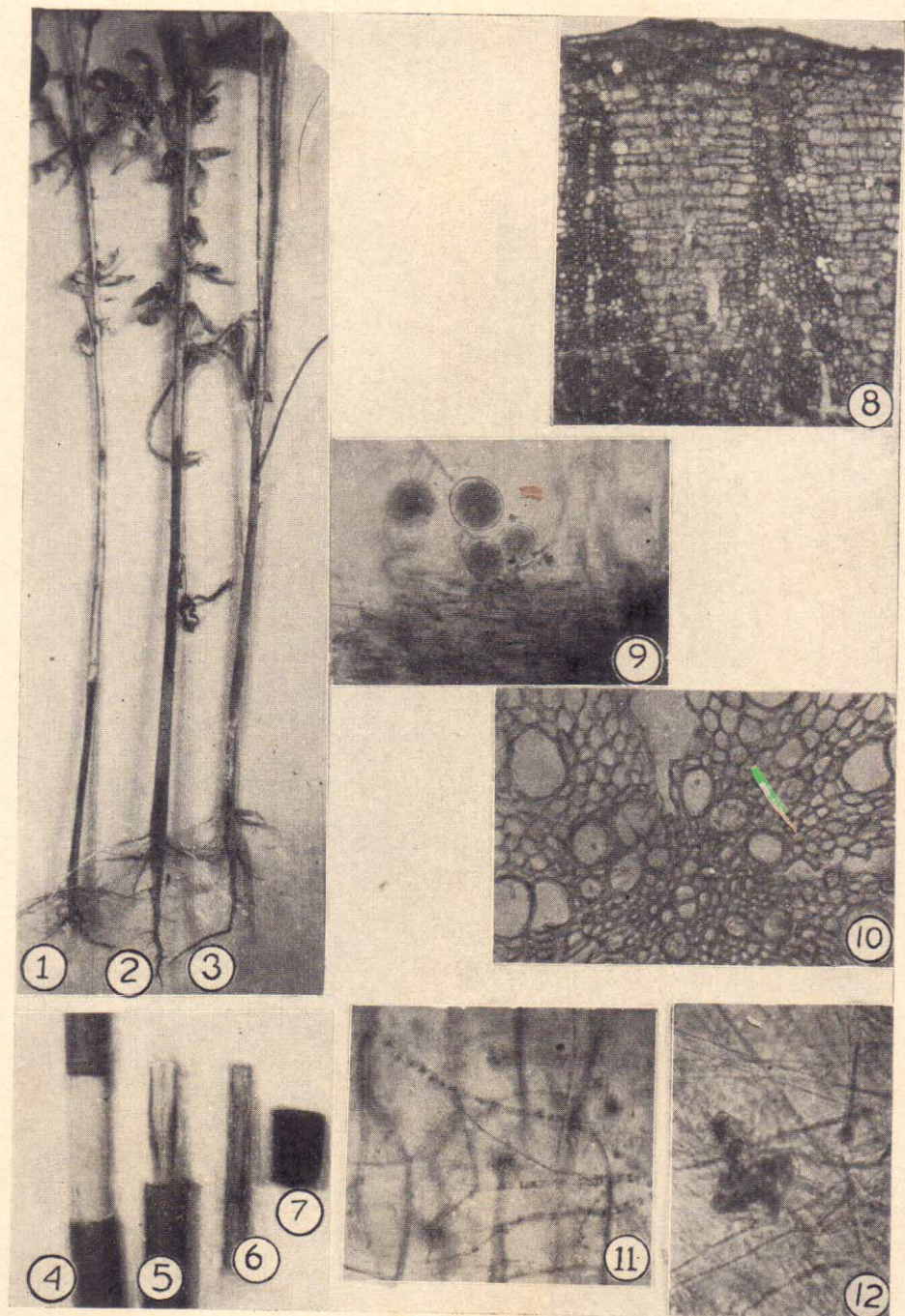
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EXPLANATION OF PLATE I

Figs. 1-12. Infected plants above four feet in height showing various stages : root-infection (fig. 1) ; foot-rot and stem-infection (figs. 2-3) ; change of colouration on the wood surface (figs. 4-7) ; cross section of the diseased stem showing hyphae in phloem rays at the initial stage of infection along with discoloured epidermal region and cells blocked by gummy depositions (fig. 8, $\times 66$) ; Sproangia, and Chlamydospores in teased materials from diseased portions of infected seedlings (figs. 9, $\times 166$) ; t.s. of root in advanced stage of infection showing hyphae in vessels and other wood-elements ; tylosis and dissolution of ray cells (fig. 10, $\times 166$) ; radial longitudinal section showing hyphae in medullary ray cells (fig. 11, $\times 166$) ; photomicrograph showing amphigynous antheridium and fertilization (fig. 12, $\times 580$).

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On *P. parasitica* var. *subdariffae* Muk.

N. Mukerjee

APPENDIX I

Showing meteorological data from the date of sowing till last date of taking records i.e., 3.6.58-3.10.58

"Meteorological data between date of sowing and first-record taken i.e. 3.6.58-30.7.58."

Rainfall in inch	Temperature °F(aveg.)						Humidity %	
	Air		Soil (depth cm)					
	Max.	Min.	Max.	Min.				
			5	15	5	15	Morn.	Noon.
14.64	93.5	78.5	97	91	84	87	91	71

"Meteorological data between second and third record, i.e., 27.8.58-17.9.58."

Rainfall in inch	Temperature °F(ave.)				Humidity %			
	Air		Soil (depth cm)					
	Max.	Min.	Max.	Min.				
			5	15	5	15	Morn.	Noon.
7.97	89	78	92	87	81	83	93	80

"Meteorological data between first and second record, i.e., 31.7.58-26.8.58."

Rainfall in inch	Temperature °F(ave.)						Humidity %	
	Air		Soil (depth cm)					
	Max.	Min.	Max.	Min.	5	15	5	15
7.86	88.5	76.8	93.6	87	81.8	82.6	92	65
							Morn.	Noon.

"Meteorological data between third and fourth record, i.e., 18.9.58-3.10.58."

Rainfall in inch.	Temperature °F(avge.)						Humidity %	
	Air		Soil (depth cm)					
			Max.		Min.			
	Max.	Min.	Max.	Min.	Max.	Min.		
			5	15	5	15	Morn.	Noon.
3.54	85.3	76.8	92	89	77.3	85	89.1	67.6