STUDIES ON *Septoria* LEAF SPOT OF FIELD BEAN (*Dolichos lablab* L.)

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Bangalore

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON *Septoria* LEAF SPOT OF FIELD BEAN (*Dolichos lablab* L.)" submitted in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY to the University of Agricultural Sciences, Bangalore, is a record of *bona-fide* research work carried out by Mr. B.N. HANUMANTHA RAJ, B.Sc(Agri), under my supervision and guidance and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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(B.N. HANUMANTHA RAJ)

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INTRODUCTION

CHAPTER I

INTRODUCTION

Dolichos lablab Linn. commonly known as field bean or avare in kannada is an important pulse and vegetable crop of Karnataka state. It is usually grown as a mixed crop with ragi (*Eleusine coracana* (Linn.) Gaertn. and as a pure crop around cities. During 1975-76 it covered an area of 62,711 ha in Karnataka with the production of 12,765 tonnes (Source : Bureau of economics and statistics, Karnataka state). The seeds are rich in protein (24.9%) and carbohydrate (60.1%) (Anonymous, 1952). Hence in the recent years considerable attention is being paid towards the development of high yielding varieties of this crop.

Several fungal pathogens are known to infect this crop. Of these leaf spot caused by *Septoria dolichi* Berk. and Curt. is of major importance. The literature available on this disease is mostly confined to the description of the pathogen and symptomatology. Information on aspects relating to the fungal metabolism and life history is lacking. Hence an attempt has been mmade to study the aspects enumerated below:

1. Morphological characters and host parasite relationship.
2. Cultural, nutritional and physiological studies.
3. Spore germination studies.

4. Disease incidence and development.

5. Post-infectional changes in the host.

6. Host range of the pathogen.

7. Perpetuation of the fungus in nature.

8. Chemical control.

The above studies were conducted during 1977-78, at Agricultural College, Bangalore. The results of the investigation are presented in the following pages.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Various species of the genus *Septoria* attack a number of plants belonging to different families. Leaf spot caused by *Septoria dolichi* is one of the important diseases of avare. This disease was regarded as of minor importance, since the severity on local crop was low and avare was of minor importance. However, with the introduction of high yielding varieties and hybrids the disease problems on this crop have also assumed importance.

Except for a brief report on the occurrence of the disease, no information is available either on the pathogen, *S. dolichi* or on the disease it causes. In view of this, all the relevant information having direct or indirect bearing on the problem investigated here, has been reviewed in the following pages.

Morphology of the fungus

Septoria dolichi was first reported by Berkeley and Curtis (Saccardo, 1884). According to them, the spores are 40 µm long and triseptate. McRae was the first to report this pathogen from India in 1909 (Sydow H and P and Butler, 1966). According to Chona and Munjal (1956) *S. dolichi* has spores 28 to 40 µm (mostly 32 to 35 µm) x 2.5 µm.

Growth on different media

Growth and cultural characteristics of a fungus such as colony diameter (dry mycelial weight) topography, sporulation etc., depend on nutrition provided by the medium. Literature on various Septoria spp. indicates that this fungus can be cultured well on non-synthetic media indicating its requirement for several nutrients.

Septoria lycopersici Speg. grows well on potato dextrose agar (Sohi and Sokhi, 1973), host extract (Patil, 1977) and on sterilised tomato leaves (Endrinal and Celino, 1940). Malt agar and celery extract agar support good growth of Septoria apiigraveolentis Dorogin. (Kochman and Kubicka, 1962). Weber (1922a, 1922b, 1923) found potato dextrose agar (PDA) as good medium for Septoria nodorum Berk., S. tritici Desm., S. secalis Prill. and Del., S. passerini Sacc., S. agropyri Ell. and Ev. and S. avenae Frank., Onion and potato media are found to support good growth of S. tritici (Ársenjevic, 1965). According to Luthra et al. (1937) S. nodorum grows well on oatmeal agar and wheat leaf decoction agar while, S. tritici grows well on onion agar. Munjal and Gautam (1977) observed maximum growth of S. humuli West. on modified Czapeck's medium.

Utilization of carbon sources

Fungi exhibit carbon heterotrophy and obtain their carbon requirement from various organic sources. Although generalization regarding carbon sources are lacking, glucose is said to be the most efficient source of carbon and energy for most of the fungi (Cochrane, 1958; Bilgrami and Verma, 1978).

Sohi and Sokhi (1973) found glucose, sucrose and maltose as good carbon sources for the growth of *Septori* *lycopersici* while glycerine was found to support least growth. Saccharose has been found to support good growth of *S. tritici* (Arsenjevic, 1966). *Septoria humuli* grows well on mannose, sucrose, and glucose followed by maltose, while glycerol supports least growth (Munjal and Gautam, 1977).

Acetate and citrate form of carbon source is not utilized by *Chytridium* spp. and *Pythiogeton* sp. (as cited in Cochrane, 1958). Several strains of *Piricularia oryzae* Cav. are also known to make no growth with sodium citrate and sodium acetate as carbon sources (Otsuka, *et al*., 1963).

Utilization of nitrogen sources

Nitrogen is an important constituent of proteins and nucleic acids. No single pattern of nitrogen assimilation can be described to apply to all fungi. Nitrates are excellent sources of nitrogen for many fungi (Lilly and Barnett, 1951). Potassium nitrate is superior over other forms of nitrate such as calcium nitrate, sodium nitrate and ammonium nitrate for a large number of fungi imperfecti (Tandon, 1967;

Mix, 1933; Suryanarayanan, 1958). However, ammonium sulphate and ammonium chloride are generally inferior to ammonium nitrate in their nutritive value (Bilgrami and Verma, 1978). Amino acids are usually good sources of nitrogen for fungi.

Munjal and Gautam (1977) recorded optimum growth of S. humuli on ammonium nitrate, sodium nitrate and urea. Further, casein hydrolysate and proline gave better growth as compared to other 13 amino acids. However aspartic and glutamic acids also supported good growth. Sohi and Sokhi (1973) found asparagine and urea as best nitrogen sources for S. lycopersici while they observed no growth in the medium, containing either asparatic acid or glutamic acid as nitrogen source. Asparagine, glycine and ammonium tartarate have been found to support good growth of S. tritici (Arsenjeric, 1966).

Effect of pH on growth

Hydrogen-ion concentration has a direct effect on the physiology of fungus. Enzyme systems, metal solubilities, entry of essential vitamins, surface metabolic reactions, uptake of minerals and entry of organic acids into the cell are all governed by pH (Cochrane, 1958). The literature available suggests that the optimum pH for growth of various Septoria spp. varies from 5.6 to 8.4.

Septoria lycopersici can grow over a wide range of pH from 4.0 to 8.5 with optimum as 6.0 for growth and 6.0 to 6.5

for sporulation (Sohi and Sokhi, 1973). According to Patil (1977) it grows over a pH range of 3.5 to 8.7 with 6.5 as optimum for growth. He observed excellent sporulation between 6.0 and 6.9. However Endrinal and Celino (1940) have noticed good growth of S. lycopersici between a pH range of 5.6 to 8.4.

Septoria tritici grows well between a pH range of 4.6 to 6.0, while S. nodorum grows well at a very wide range of pH from 3.0 to 8.3 (Luthra et al., 1937). Weber (1922a and 1922b), noted 6.2 as the optimum pH for the growth of S. avenae and good growth of S. tritici between a pH range of 3.8 to 8.0 while both the fungi could grew over a range of pH from 2.5 to 9.2.

Septoria humuli can grow over a wide range of pH from 4.0 to 9.0 with an optimum of 6.0 for growth and 5.5 for sporulation (Munjal and Gautam, 1977).

Temperature requirements

Temperature affects growth, spore germination, reproduction and indeed all activities of the organism. The literature available indicates that the optimum temperature for growth of Septoria spp. ranges from 18 to 28°C.

The optimum temperature for growth and sporulation of S. lycopersici is 25°C. Further it grows and sporulates poorly at 10°C and 35°C (Patil, 1977). Sohi and Sokhi (1973) also found 25°C to be the optimum temperature for growth and 20 to 28°C, for sporulation. They found 10°C and 28 to 30°C as minimum and maximum temperaturesfor growth, respectively. According to Pritchard and Porte (1924), 25°C is optimum for growth and sporulation of S. lycopersici. They found 2°C and 34.5°C as minimum and maximum temperatures for growth respectively. Corresponding figures for sporulation were 15°C and 27°C. S. nodorum has an optimum temperature requirement of 25°C (Luthra et al., 1937). However, Weber (1922) states that, it ranges from 20 to 24°C with 4°C and 32°C as minimum and maximum temperatures, respectively.

The optimum temperature for growth of S. tritici ranges from 20 to 26°C with 2°C as minimum and 36°C as maximum temperatures for growth (Weber, 1922b). Arsenjevic (1965) reported 22 to 26°C as optimum temperature for the growth of S. tritici while Luthra et al. (1937) found it to be 25°C. The minimum, optimum and maximum temperatures for growth of S. avenae are 2°C, 20 to 25°C and 32°C, respectively (Weber, 1922a). According to Munjal and Gautam (1977) S. humuli grows over a wide range of temperature from 5°C to 35°C with 20 to 25°C as best for growth and sporulation. Further, less growth was recorded at 30°C and 5°C and least at 35°C.

Effect of light on growth and sporulation

Light is absolutely required for the formation of various types of reproductive organs in certain species of fungi (Cochrane, 1958). Richards (1951) observed that cultures of *S. nodorum* kept in continuous darkness failed to sporulate while he recorded abundant sporulation in cultures under continuous light of atleast 100 ft candles. *Septoria lycopersici* produced greater number of cirri under constant illumination or alternate light and darkness cycles than in constant darkness (Kurozawa and Balmer, 1975).

Spore germination studies

Spores are the principal agents of dispersal of fungi. Consequently any consideration of ecology or of the spread of economically important fungi will have to take into account the spore germination. Nutritional dependance of spore germination is very well demonstrated in several fungi (Cochrane, 1958). According to Patil (1977) spores of *Septoria lycopersici* germinate well in leaf decoction while less in tap water. Spores of *S. tritici* germinate rapidly in wheat leaf decoction (Luthra *et al.*, 1937). However, it has been observed that both *S. tritici* and *S. nodorum* germinate well in water (Shipton *et al.*, 1971).

Temperature has got profound influence on spore germination. Literature on this indicates that optimum temperature for spore germination of several *Septoria* spp. varies from 11.5°C to 27°C.

The optimum temperature for spore germination of *Septoria passerini* has been found to be 20 to 24°C (Green and

Dickson, 1957). Luthra et al. (1937) found 20°C as the optimum temperature for spore germination of S. tritici, with the maximum ranging between 25 to 30°C and minimum below 5°C. However, Weber (1922b) found 22 to 26°C, 2 to 3°C and 32°C as optimum, minimum and maximum temperature respectively for spore germination of S. tritici. According to Chona and Munjal (1952) spores of S. nodorum germinate well between 20 to 22°C and do not germinate either b below 12°C or above 30°C. Further, Weber (1922b) reported 20°C as best temperature for spore germination of S. nodorum. The minimum, optimum and maximum temperatures for spore germination of S. lycopersici are 3°C, 25°C and 30°C, respectively (Sohi and Sokhi, 1973). However, MacNeil (1950) observed optimum germination of spores of this fungus at temperatures between 21 to 29°C with no germination at 31°C. The optimum temperature for spore germination of S. apii and S. appiigraveolentis has been found to be 17 to 27°C and 11.5 to 25.5°C, respectively(Cochran, 1932). Schnieder (1959) reported 0 to 1°C as minimum temperature for spore germination of both S. obesa and S. chrysanthemella Sacc. while maximum as 31°C for the former and 32°C for the latter. Optimum temperature for spore germination of S. avenae ranges between 20 to 24°C and no germination occurs above 36°C. At 4°C germination is prolonged (Weber, 1922a).

Available water is one of the primary determinants of spore germination. Most of the fungi reuire a high relative humidity (RH) for spore germination with the well known exception of powdery mildews. Sheridan (1968) observed spore germination of Septoria apiicola at 96.8 per cent and above, but not at 96 per cent RH. The minimum relative humidity requirements for spore germination of S. obesa and S. chrysanthemella are 92 per cent and 91 per cent, respectively (Schnieder, 1959).

Effect of oil on fungus

Various types of oil have been shown to control several plant diseases and to inhibit many phytopathogenic fungi. The effect of oil on sporulation, germination and stomatol penetration by Mycosphaerella musicola is in dispute. There is general agreement that oil has a therapeutic action after the fungus penetrates into the leaf or during early sumptom formation. In Septoria leaf spot of celery there is evidence for two modes of oil action: (1) drastic lowering of sporulation, and (2) an inhibition of infection or of symptoms. However, no further details are available (Wilson, 1961). Calpouzos (1966) who has reviewed a literature on oils feels that oil sprays may shift the physiology of leaf tissue which might result in disease resistance.

Conditions favouring infection and disease development

Environmental factors, particularly temperature, humidity and rainfall have significant effect on infection and development of disease.

MacNeil (1950) found that a minimum of 48 hrs 100 per cent RH is required for germination and penetration of tomato leaves by Septoria lycopersici. It has been observed that severe damage by this fungus occurs in the parts of U.S.A., in a temperature belt of 23°C to 25.5°C (Pritchard and Porte, 1924). According to Endrinal and Celino (1940), it is more destructive under humid conditions. A temperature of 20 to 25°C with 75.6 to 92.9 per cent RH were congineal for the disease development (Sohi and Sokhi, 1969). However they found no correlation between the disease incidence, RHand rainfall, but temperature played a major role in disease development. S. lycopersici spreads widely during wet weather but checked by a period of prolonged dry weather (Reed, 1911).

Arsenjevic (1965) found that rainy weather, early sowing and high doses of fertilizers favour the infection of wheat by S. tritici. Further, a minimum of 20 hrs high humidity at 12°C is required for infection (Holmes and Colhoun, 1974). Studies under controlled conditions and in field by Scharen (1964) indicate that onset of infection by Septoria nodorum on new leaves and heads of wheat, depends upon (a) rain and wind with splashing water droplets to release and distribute spores and (b) a subsequent humid period of atleast 48 hrs during which germination, penetration and successful establishment of the fungus occur. However, Holmes and Colhoun (1974) claim that only 3 hrs high humidity at 12°C is sufficient for S. nodorum to attack wheat plants.

Septoria passerini develops more rapidly on barley between a temperature range of 20 to 24°C (Green and Dickson, 1957). Buddin and Wakefield (1924) observed that Septoria sp. attacking antirrhinum is favoured by cool and moist weather.

Mode of penetration

Septoria spp. enter their hosts either directly or through stomata or both.

The entry of Septoria lycopersici has been found to be by direct penetration as recorded by Patil (1977) and Chupp (1925) while Endrinal and Celino (1940) observed only stomatal penetration of S. lycopersici. Sohi and Sokhi (1973) also support the latter view. The entry of S. nodorum and S. tritici has been found to be by direct penetration (Weber, 1922a, 1922b), while Arsenjevic (1965) has reported the entry of S. tritici to be chiefly stomatal. S. apii enters celery leaves either through stomata or more commonly through the epidermal cells (Campanile, 1926). Green and Dickson (1957) report the penetration of S. passerini to be mainly through stomata. However they also found evidence for penetration between the epidermal cells. Septoria glycines Hemmi. enters soybean leaves through stomata (Wolf, 1926). Corey (1962) observed definite penetrations of stomata by S. linicola (Speg.) Gar., and noted apparent direct penetration also.

Histopathology

Pathogenic fungi induce several histologic changes in their host. Cunningham (1928) while studying the histological changes induced by certain leaf spotting fungi observed many changes due to infection. In the lesions produced by *Septoria conspicus* Ellet.&Mart. on *Steironema ciliatuma* (Linn.) Raf., the diseased portion was comparatively thinner than the healthy tissues of the leaf. In holonecrotic area, the epidermal cells had collapsed. Palisade mesophyll cells and spongy parenchyma were completely filled with dense granular substance. In the lesions produced by *Septoria apii* on celery, cells were killed with their contents disappearing and the walls collapsed. The necrosis shaded off gradually into healthy tissues without the appearance of a cork cambium.

Green and Dickson (1957) while working with *Septoria passerini* on Barley observed sparse intercellular hyphae without haustoria in the leaves, which incited necrosis of mesophyll cells, but did not normally attack other tissues of the leaves. Thickening of the cell walls in contatt with hyphae was observed. Further shrinkage of cell contents without intercellular deposits was also observed which they suspected to be due to loss of water by the cells as the disease progressed.

Ramification of leaf tissue by the fungus Septoria linicola with collapsing of mesophyll cells. The mesophyll cells were apparently weekened in advance of the fungus as some collapsed beyond the margins of the infected area. S. linicola after entering into the host leaf rainfed the tissue and mesophyll cells collapsed. Further, mesophyll cells in advance of the fungus were apparently weakened. At the margins of the infected area, the mycelium was frequently appressed to the cells of host, but no haustoria or intra-cellular mycelium was observed (Corey, 1962).

Post-infectional changes in the host

Several changes in the host are induced by the pathogens after infection.

Changes in phenol contents:

Phenolic compounds have been implicated in many functions of plant. They play a role in defense reactions (Pridham, 1960) and in wound healing (Samuel, 1927 and Kasuge, 1969).

Changes in phenol metabolism appears to be a common phenomenon in the diseased plants. Increase in total phenol content has been observed in wheat leaves infected with stem rust (Kiraly, 1962). Raghunathan et al. (1966) observed an increase in phenol content of fruit peels of banana soon after infection by Gloeosporium musarum Cke.. A steep increase of phenols in citrus plants infected by Xanthomonas citri (Hasse.) Dowson has been reported by Kishore and Chand(1975).

Changes in amino nitrogen:

The content of amino nitrogen is indicative of the soluble nitrogen status of the plant tissue. Thus an increase in its content might indicate a rapid degradation of proteins or a synthesis of amino acids.

McCombs and Winstead (1964) observed decreased in levels of amino acids except -alanine, two days after infection of cucumber fruits by Pythium aphanidermatum (Edsm.) Fritz.. Then the concentration increased by the 4th day after inoculation. They attributed this increase to proteolysis of existing tissue proteins or synthesis of amino acids by the fungus. Raghunathan et al. (1966), noticed an increase in concentration of 9 amino acids in banana fruit coat soon after inoculation with Gloeosporium musarum but declined after 120 hrs.

Perpetuation of the pathogen

There is considerable variation in the longevity of Septoria spp. in culture as well as in the diseased leaves.

Septoria lycopersici can survive upto seven months in diseased leaves under laboratory conditions (Patil, 1977), atleast upto six months on dried infected leaves (Endrinal and Celino, 1940). Sohi and Sokhi (1973) found it to over winter in the soil under the conditions prevailing at 'Solan' on host debris. S. nodorum can survive for more than 18 months in the form of spores (Weber, 1922b) while S. tritici can survive for 10 months on wheat leaves. Mande and Shuring (1970) found the survival period of S. apiicola on diseased celery debris in soil to be upto 9 months.

Chemical control

In-vitro evaluation of fungicides:

Response of a fungus to different fungicides varies. In-vitro evaluation of fungicides provides a rapid and easiest technique of screening fungicides. These results need to be confirmed under field conditions. Patil (1977) found thiram as the best fungicide followed by Bavistin and Captan against S. lycopersici in-vitro.

Field evaluation of fungicides:

Results obtained by various workers suggest that no single fungicide is effective against all the Septoria spp.

Excellent control was obtained by captafol against S. apiicola, while Benomyl and Bavistin gave poor results (Wilson, 1974). Nine years data from Ontario Agricultural experimental farm indicated that only Bordeaux mixture could control S. apii (Anonymous, 1922). Difolatan was found to be the best among five fungicides tested in controlling fruit spots of grapes caused by Septoria spp. (Fouget and Gonzalez (1976). Whetzel (1922) got good control of S. lycopersici with Bordeaux mixture with the addition of fish oil soap. Sohi and Sokhi (1970) also obtained best control with Bordeaux mixture 4:4:50 and Dithane Z-78 (0.2%). They have recommended weekly sprays of Dithane Z-78 for effective control of the disease considering the economics of spraying and yield of crop. Miller and Lin (1954) while reviewing the literature on the efficacy of fungicides in the control of certain genera of plant pathogenic fungi observed that copper fungicides were better than the organic compounds against several Septoria spp.

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

Collection, isolation and maintenance

The fungus was isolated from the diseased leaves of Dolichos lablab, collected from the experimental fields of Main Research Station, Hebbal, Bangalore during May 1977. The fungus was isolated on potato dextrose agar (PDA) by the common tissue isolation technique by disinfecting the leaf bits containing diseased portion in sodium hypochlorite for two minutes. After washing the bits in sterile water for several times, they were transferred on to slants containing PDA. In order to eliminate the possible contamination of fast growing saprophytes the slants showing fungal growth during first 6 days were discarded and the remaining were retained with regular observations. The growth obtained in such PDA slants were taken for artificial inoculation after observing them under light microscope and confirming their identity as Septoria spp.,. The growth on PDA slants was crushed into fine suspension and this suspension was automized on to the leaves of avare, previously washed with sterile distilled water. All the plants were kept in humid chamber and observations were made regularly. When the leaves showed typical disease symptoms, they were collected and the spore suspension was obtained by keeping them in sterile water to which a pinch of streptomycin was added.

A drop of this spore suspension was placed on one per cent water agar in petridishes and spread using a bent glass rod. The isolated single spore was marked and using a cork borer it was lifted and transferred on to a petridish containing PDA. Out of several such isolations, only one was selected and multiplied, discarding the others. The fungus was transferred to several PDA slants which were kept in refrigerator at 4°C, after considerable growth was observed. This culture was used in all the studies and the fungus was renewed by subculturing once in two months.

Glasswares and chemicals

In all experiments on growth studies only acid washed Corning glasswares and Analar grade BDH, Oxoid and Difco chemicals were used.

Sterlisation

All the media and glasswares were sterilised by autoclaving for 15 minutes at 15 psi unless otherwise stated.

Method of inoculation

All the liquid media were inoculated as follows:

A disc of 0.5 mm diameter was taken using cork borer from the edge of actively growing culture. The lump was placed in 5 ml sterile distilled water and crushed into a fine suspension. This was used as a standard inoculum. Inoculations were done by diluting suitably and 0.2 ml of the suspension was added to each flask using sterile one ml pipette.

Petridishes containing solid media were inoculated using an inoculation needle with a loop of 3 mm diameter.

Measurement of growth

In all studies on liquid media the growth was measured by taking dry mycelial weight. This was done as follows.

Mycelial growth was harvested in 9 cm Whatman No. 42 filter paper discs which were previously dried at 60°C and weighed to a constant weight. The dry weights were taken after harvesting the myceliam and drying in hot air oven at 60°C for 48 hours.

In studies on solid media colony diameter was measured.

Preparation of different media

The composition and method of preparation of media used in the studies are given below. Most of the media were prepared by following the procedure given by Tuite (1969) and Riker and Riker (1936).

1. Potato dextrose agar:

Peeled potatoes	200 g
Dextrose	20 g
Agar agar	20 g
Distilled water	1 l

2. Richards' medium:

Potassium nitrate (KNO_3)	10 g
Potassium dihydrogen phosphate (KH_2PO_4)	5 g
Magnesium sulphate ($MgSO_4$-$7H_2O$)	2.5 g
Ferric chloride ($FeCl_3$-$6H_2O$)	0.02 g
Sucrose	50.00 g
Agar agar	20.00 g
Distilled water	1 l

3. Modified Cox's medium:

Magnesium sulphate ($MgSO_4$-$7H_2O$)	0.75 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.25 g
Aspargine ($C_4H_8N_2O_3H_2O$)	2.00 g
Glucose	2.00 g
Agar agar	20.00 g
Distilled water	1 l

4. Leonian medium:

Peptone	0.625 g
Maltose	6.25 g
Potassium dihydrogenphosphate (KH_2PO_4)	1.25 g
Malt extract	6.25 g
Magnesium sulphate ($MgSO_47H_2O$)	0.625 g
Agar agar	20.0 g
Distilled water	1 l

5. Asthana and Hawker's medium:

Glucose	5.00 g
Potassium nitrate (KNO_3)	3.50 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.75 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.75 g
Agar agar	15.00 g
Distilled water	1.00 l

6. Sabouraud dextrose agar:

Dextrose	40.00 g
Peptone	10.00 g
Agar agar	20.00 g
Distilled water	1.00 l

7. Host leaf extract agar:

Avare green leaves (*D. lablab*)	200 g
Agar agar	20 g
Water	1 l

The leaves were boiled for 30 min. and then strained through cheese cloth. Volume was made upto one litre and agar was added.

8. Avare (*D. lablab*) pod extract agar:

Avare green pods	60 g
Agar agar	20 g
Water	1 l

Pods were cut into pieces and boiled for 15 min. and then strained through cheese cloth. Volume was made upto one litre and agar was added.

9. <u>Avare seed (*D. lablab*) extract agar:</u>

Avare seeds	60 g
Agar agar	20 g
Distilled water	1 l

Seeds were boiled for 15 min. and strained through cheese cloth. Volume was made up to one litre and agar was added.

10. <u>Tochinai's medium:</u>

Potassium nitrate (KNO_3)	2 g
Sucrose	30 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.1 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.5 g
Potassium monohydrogen phosphate	0.5 g
Ferric chloride	Trace
Agar agar	20.0 g
Distilled water	1.0 l

11. A mixture of leaf extract, seed extract, and PDA in equal proportions.

Morphological studies

Morphology of the fungus in culture as well as in the host was studied. Fifteen days old growth on PDA was used. Length and breadth measurements of conidia, pycnidia and hyphae were taken using caliberated ocular micrometer. Number of septa in a conidium were also noted.

Growth of the fungus in different solid media

Eleven different solid media described above were prepared and 20 ml of each medium was poured into a 3 inches petriplate and the fungus was inoculated. All the petriplates were incubated at room temperature. After 30 days the colony diameter was measured and other cultural characteristics such as sporulation, colour and topography of colony, etc., were recorded. The results obtained were statistically analysed.

Growth phase of the fungus

Growth phase of the fungus was studied in potato dextrose broth. Ten ml of PDB was added to 50 ml conical flasks and sterilised. All the flasks were inoculated and incubated at 25 ± 1°C. The harvest of mycelial growth was made on 8th day and subsequent harvests were done at 5 days interval, with five replications. In each case dry mycelial weights were recorded. The experiment was continued upto 43rd day only since the last two consecutive dry weights showed reduction in mycelial dry weight.

Growth of the fungus in different liquid media

Ten different liquid media viz., potato dextrose broth, *Tochina's medium, Asthana and Hawker medium*, modified Cox's medium, Richards' medium, Leonian medium, pod extract, Sabouraud dextrose medium, seed extract, and leaf extract, were prepared as described previously.

Twentyfive ml of each medium was poured into 100 ml conical flasks and replicated thrice. All the flasks were inoculated and incubated at 25 ± 1°C. After 30 days, the growth was harvested and dry mycelial weights were recorded

Nutritional studies

Utilization of different carbon sources:

The utilization of different carbon sources by Septoria dolichi was studied in Tochinai's medium. Ten carbon sources viz., dextrin, glucose, glycerol, lactose, mannitol, rhamnose, sodium acetate, sodium citrate, soluble starch and sucrose were tried. The quantity of each carbon compound to be added was determined on the basis of their molecular weights so as to provide equivalent amount of carbon as was provided by 30 g sucrose in the Tochinai's medium. Twenty ml of each carbon source containing basal medium was added to a 50 ml conical flask and replicated five times. All the flasks along with the medium were sterilised for 10 min. at 10 lb pressure.

The flasks were inoculated with the fungus and incubated at 25 ± 1°C. After 30 days incubation, growth was harvested and dry mycelial weights were recorded.

Utilization of different nitrogen sources:

The utilization of ten different nitrogen sources viz., asparagine, asparatic acid, ammonium nitrate, ammonium sulphate, alanine, glutamic acid, lysine, potassium nitrate, proline and tryptophan, was studied in Tochinai's medium. The quantity of each nitrogen compound to be added was determined on the basis of its molecular weight so as to provide equivalent quantity of nitrogen as provided by 2 g of potassium nitrate in Tochinai's medium. The quantity of carbon provided by each organic nitrogen source was determined and accordingly the quality of sucrose was reduced. Twenty ml of each nitrogen compound containing basal medium was poured into 100 ml flasks and replicated four times. All flasks were sterilised, inoculated with the fungus and incubated at 25 ± 1°C. After 30 days incubation, growth was harvested and dry mycelial weights were recorded.

Physiological studies

Effect of pH on growth of the fungus:

Effect of hydrogen-ion concentration on the growth of Septoria dolichi was studied in Tochinai's medium. Citric acid and phosphate buffers at different pH levels were prepared as per the schedule given by Vogel (1953), which is given under appendix. One hundred ml of buffer at 12 different levels viz., 2.4, 3.0, 3.6, 4.0, 4.6, 5.0, 5.6, 6.0, 6.6, 7.0, 7.6 and 8.0 were used. The ingredients of the medium were weighed for 1800 ml of the medium and dissolved in 600 ml distilled water. Fifty ml of this was transferred to 250 ml conical flasks containing 100 ml of buffers, Twentyfive ml of this mixture was dispensed into six, 100 ml conical flasks and autoclaved. After autoclaving, only five flasks in each treatment were inoculated with the fungus and were incubated at 25 ± 1°C. After 30 days the growth was harvested and dry mycelial weights were recorded. The pH of the medium after sterilisation was noted using the sixth flask.

<u>Temperature studies</u>:

Sabouraud dextrose agar was used for studying the temperature requirements of <u>S</u>. <u>dolichi</u>. Eight temperatures viz., 0, 5, 10, 15, 20, 25, 30 and 35°C were tried.

Twenty ml of sterilized Sabouraud dextrose agar was poured into each 3 inches petriplates. After solidification of the medium, the fungus was inoculated and incubated at different temperatures and was replicated three times. After 20 days the colony diameter was measured and observations on sporulation were taken.

Effect of light on growth and sporulation

The effect of light on sporulation of S. dolichi in culture was studied on Sabouraud dextrose agar. About 15 ml of sterilised Sabouraud dextrose agar was poured into 3 inches petriplates and were inoculated with the fungus. All the inoculated petridishes were incubated at room temperature and exposed to different durations of light viz., 24 hrs exposure to light, 12 hrs light, and 12 hrs darkness, and complete darkness. After 20 days the colony diameter was measured and observation on sporulation was recorded.

Spore germination studies

"Hanging drop" method was employed in the studies on spore germination. A drop of spore suspension was placed on a clean coverslip, which was inverted and placed over a cavity slide. The edges of coverslip were sealed using vaseline. The slides were placed in petridishes lined with moistened absorbant cotton.

(a) Spore germination in different media:

The germination of spores was studied in different media viz., host extract, sucrose solution (1%), glucose solution (1%), distilled water and tap water. Host extract was prepared by boiling 10 g of avare leaves in 100 ml water for 10 min. and then straining through a cheese cloth. All the slides were kept at room temperature and germination counts were taken after 24 and 48 hrs. Percentage germination was calculated by counting the germinated and ungerminated spores in 5 microscopic fields per slide for three replications.

(b) <u>Spore germination at different temperatures:</u>

Spore germination at different temperatures viz., 0, 5, 10, 15, 20, 25, 30 and 35°C was studied in host extract. The percentage germination was recorded as above after 48 hrs.

(c) <u>Spore germination at different relative humidities:</u>

Spore suspension (10^4/ml) was prepared in host extract and a loopful of this was placed on a clean cover glass and allowed to dry completely. Then these cover glasses were placed in closed containers, kept at 25 ± 1°C maintaining different relative humidities. After 48 hrs a small drop of lactophenol was placed on each cover glass, which was inverted over a slide. The germination counts were taken and per cent germination was recorded.

The different levels of relative humidity (RH) were maintained by using following saturated salt solutions (Winston and Bates, 1960).

Sl. No.	Saturated solution	Relative humidity(%)
1.	Potassium dichromate	98.0
2.	Potassium dihydrogen phosphate	96.0
3.	Potassium nitrate	92.5
4.	Zinc sulphate	88.5
5.	Potassium chloride	85.0
6.	Sodium chloride	75.5

Water was used to create 100 per cent relative humidity.

Effect of relative humidity on infection

In this experiment pot cultured 30 days old HA-3 plants were used. The leaves of each plant were inoculated with the spore suspension (10^4/ml) using an automizer till few drops of suspension fell from the leaf. The plants were covered with polythene bags to provide 100 per cent RH. At an interval of 6 hrs the polythene bags were removed and the process was continued upto 48 hrs. In each case three plants were used. After 8 days the plants were regularly observed for symptoms.

Disease development

In this experiment 3 x 3 m^2 plots were selected and sown with Hebbal avare-3 (Ha-3) at an interval of 15 days.

Following observations were recorded at fortnightly interval. Ten plants in each plot were selected randomly, numbered and tagged. Number of leaves per plant and number of diseased leaves were counted and noted. Each diseased leaf was numbered and tagged. Severity of infection/disease on each leaflet of the diseased leaf was also recorded. Severity was recorded, based on the disease intensity charts, which were prepared after several observations of the diseased leaves and calculating the diseased area per leaf using graph sheets.

Temperature, RH and rainfall data for the period were collected from Metereological observatory, Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore.

Mode of penetration

Mode of penetration of S. dolichi was studied by using the method of Diener (1955). Thirty days old avare plants were selected and circles of about 5 mm diameter were marked using Indian ink on upper surface of leaves and a loopful of spore suspension of 10^4 concentration was placed inside the circle on the leaf. It was allowed to dry and then plants were transferred to humid chamber. At an interval of 24 hrs the marked leaves were plucked and the portion enclosed by the circle were cut and placed in a mixture containing equal parts of glacial acetic acid and 95 per cent ethyl alcohol. After two days, the bits were transferred to 75 to 80 per cent lactic acid. After 4 days each bit was placed on a seperate slide in a drop of 0.1 per cent cotton blue in lactophenol with the marked surface upwards. After 5 min. the excess was removed and clear lactophenol was added. A coverslip was placed on the bit and observed under light microscope.

Histopathological and histochemical studies

Histopathological and histochemical studies were made using the healthy, stage-I (Plate 2a) and stage-II (Plate 2c) leaf bits.

Fixation and dehydration:

The leaf bits (10 x 3 mm) were killed and fixed in Carnoy's B fixative (6 parts ethyl alcohol + 3 parts of chloroform + 1 part of acetic acid). They were later washed in 80 per cent alcohol for 15 min. and subjected to dehydration using absolute alcohol - butanol grades at 3:1 and 1:1 proportions and then treated with pure butanol, twice.

Tissue infiltration and embedding:

The materials were transferred from the medium of pure butanol to small vials and chips of paraffin were added successively until the medium reached a saturation point at the room temperature and later under the table lamp (40 watts). Finally, the materials were given 5 to 6 changes with the molten pure paraffin in the oven at 60°C, thus replacing the last traces of butanol with paraffin. The materials were then embedded in paraffin employing paper boat method.

Microtoming:

Serial microtome sections of 8 µm thickness were obtained using A.O. spencer microtome.

Affixing the sections to slides:

Gelatin (0.2 per cent) with a little potassium dichromate was used as an adhesive.. Xylol was used to deparaffinise the sections. Then the slides were passed through the solutions butanol and then ethanol series successively for hydration. After staining the sections were dehydrated passing through ethanol butanol series and mounted in DPX.

Histochemical staining:

Histochemical assessment was made for insoluble polysaccharides, nucleic acids and proteins. Following table gives the details of the histochemical procedures adopted in the present investigation.

After staining, the sections were observed under a light microscope, observations were recorded and photomicrographs were also taken.

Metabolite	Tests (from Jensen, 1962)	Indication
Insoluble poly-saccharides	Periodic acid-Schiff's Test (PAS) (Hotchkiss, 1948)	Magenta colour
Starch	Iodine-Potassium iodide test (Johansen, 1940)	Brownish violet or deep blue
Proteins	Mercuric bromophenol blue method (Mazia *et al*., 1953)	Deep blue
Nucleic acids	Azur B method (Flax and Himes, 1952)	DNA-greenish RNA-purple or deep blue
	Methyl green-pyronin (MGP)	DNA-green or deep blue RNA-deep purple.

Biochemical changes due to infection

Extraction of the plant sample:

Two grams of the fresh leaf material sampled from both diseased (about 20% affected) and healthy plants were used for chemical analysis. Each sample was cut into small bits and suspended for 5 min. in about 20 ml of 80 per cent boiling ethanol held in a hot water bath, after which it was ground well in a glass pestle and mortar for 10 min. and filtered through cheese cloth (Radhakrishnan *et al*., 1955). The residue was re-extracted with about 10 ml of 80 per cent hot ethanol and filtered as described earlier. Both extracts were pooled and cleared by filtering through Whatman No. 42 filter paper. The final volume was reduced to 5 ml in a flash evaporator under reduced pressure at 50°C, thus to represent 0.4 g of plant material (fresh) by one ml portion of the extract.

Estimation of total phenols:

Total phenols were estimated by the method of Bray and Thorpe (1954). To one ml of the extract one ml of FCR and 2 ml of 20 per cent Na_2CO_3 were added and heated on a boiling water bath exactly for one min. The resultant blue color was diluted to a known volume and read at 515 nm in a spectronic-20 (Bausch and Lamb) colorimeter. Appropriate blanks were prepared using distilled water and necessary adjustments to 100 per cent transmission were made with this. A standard curve was prepared with catechol. The quantity of total plants were expressed as mg/g of fresh plant material.

Ortho-dihydroxyphenols (O.D.Phenols):

O.D.Phenols were estimated by the method of Johnson and Schaal (1957). To one ml of the extract, one ml of Arnow's reagent, one ml of 0.5 N HCl and 2 ml of one N NaOH were added. The pink colour developed was diluted to a known volume and was read at 540 mm in a spectronic 20 colorimeter. Standard curve was prepared using catechol and the quantities of O.D.phenols were expressed in mg/g of fresh leaf material.

Estimation of free amino nitrogen:

Free amino nitrogen was estimated by the method of Spies (1957). The sample (0.5 ml) was placed in a photometer tube, and 1.0 ml of ninhydrin solution was added. The tube was covered with an aluminium cap and the contents mixed. A rack of tubes was heated for 20 min. in a boiling water bath. Five ml of diluent was then added to each tube and the contents mixed. The tubes were wiped, shaken and transferred to a dry rack. Readings were taken on a spectrophotometer at 570 nm starting 15 min. after the tubes have been removed from the water bath. Appropriate correction for blank was made by zeroing the instrument on the blank reading obtained. A standard curve was prepared with leucine.

Perpetuation of the fungus

Survival of S. dolichi in the diseased leaves and also in culture was studied. Infected leaves were collected and stored at room temperature in the laboratory in paper bags after pressing and drying them properly. At a regular interval of 15 days survival of the fungus was studied by keeping the spores collected from these leaves for germination in host extract.

Culture on PDA slants was stored at room temperature and tested for viability at an interval of 15 days by transferring a bit of culture on to a fresh PDA slant.

Host range studies

In this study nine commonly cultivated legumes and tomato, on which *Septoria* leaf spot is severe, were tested for pathogenicity by *S. dolichi*.

Sl. No.	Common name	Scientific name	Variety
1.	Cowpea	*Vigna unguiculata* (L.) Walp.	C-152
2.	Horse gram	*Dolichos uniflorus* Lam.	Hospet 1607
3.	Soybean	*Glycine max* (L.) Merr.	EC 39824
4.	Beans	*Phaseolus vulgaris* R.	Pinto
5.	Peas	Pisum sativum L. sens sample	Newlin perfection
6.	Groundnut	*Arachis hypogea* L.	HG-8
7.	Green gram	*Phaseolus aureus* L.	Sheela
8.	Black gram	*Phaseolus mungo* L.	Krishna
9.	Tomato	*Lycopersicon esculentum* Mill.	Pusa ruby
10.	Tur	*Cajanus cajan* L.	HY-3C

Thirty days old potted plants were inoculated by automizing the spore suspension (10^5/ml) on to both the surfaces of leaves. In each case three plants were used and all the plants were kept in humid chamber, after inoculation periodic observations were made upto 30 days after which it was discontinued.

Evaluation of fungicides

(a) In-vitro evaluation:

In this study, following five fungicides were tested against *S. dolichi* using poisoned food technique.

Sl. No.	Trade name	Chemical name	Concentration (%)
1.	Dithane Z-78	75 per cent zinc ethylene bisdithiocarbamate	0.1, 0.2 and 0.3
2.	Dithane M-45	Zinc and Manganese salt of ethylene bisdithiocarbamate	-do-
3.	Blitox	Copper oxychloride	-do-
4.	Difolatan	N-(1, 1, 2, 2-tetrachloroethyl sulfemyl)-Cis-Le-Cyclohexene-1, 2-dicarboximide	-do-
5.	Bavistin	[2-(Methoxy-carbomyl) Benizimimidazole]	0.05, 0.1 and 0.2

The stock solution (10%) of above fungicides was prepared in sterilised distilled water. Sabouraud's dextrose agar was prepared in 250 ml flasks. To each flask containing medium requisite quantity of stock solution of fungicide was pipetted, so as to get the required concentrations. The fungicide was mixed thoroughly by shaking the flask. Then the medium was poured into 3 inches petriplates. After solidification, the fungus growing on PDA was cut into small discs of 3 mm diameter and placed in the centre of each petridish aseptically. Petriplates containing only the medium were also inoculated. Four replications were maintained in all the cases. After 20 days of incubation, the colony diameter was measured.

(b) Field evaluation of fungicides

The same five fungicides were used in the field to know their performance under field conditions. All the fungicides were used at 0.2 per cent concentration except Bavistin which was used at 0.05 per cent. Hebbal avare-3 was sown in plots of 3 x 3 m^2 in RCBD design with four replications. The first spray was given when symptoms of disease appeared in the field and the second and third sprays were given at an interval of 15 days. Seventy five days old crop was evaluated by taking 10 plants in each plot and observations were recorded as follows. Total number of leaves/plant, number of diseased leaves/plant and percentage leaf area destroyed per plant.

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

A leaf spot disease of *Dolichos lablab* caused by *Septoria dolichi* was found to be severe in several fields. Since there was very little information available on certain aspects of the pathogen and disease, studies were undertaken which included the following: Isolation and pathogenicity, morphological, cultural, physiological and nutritional studies, spore germination studies, disease development, histopathology and histochemistry of diseased leaves, biochemical changes in the leaves due to infection, host range, perpetuation of the pathogen and chemical control. The results obtained are presented in this chapter.

Morphology of the fungus

Morphological studies of the pathogen were made both from culture and host.

From culture:

The mycelium consisted of two types of hyphae; brown and hyaline. Brown hyphae were thick and short celled. While hyaline hyphae were thin and long celled, brown hyphae measured 6.30-15.75 µm length x 3.15-5.25 µm thick breadth, with an average of 11.99 x 4.49 µm. Pycnidia, pycnidial initials were mainly made up of such brown hyphae. Hyaline hyphae were

Plate 1A. Photomicrograph showing spores of *S. dolichi* (X 400).

Plate 1B. Photomicrographs showing the different stages in the development of pycnidium in the host.

a) Aggregation of hyphae below the epidermis.

b) An advanced stage of aggregation.

c) Young pycnidium with the host epidermis intact.

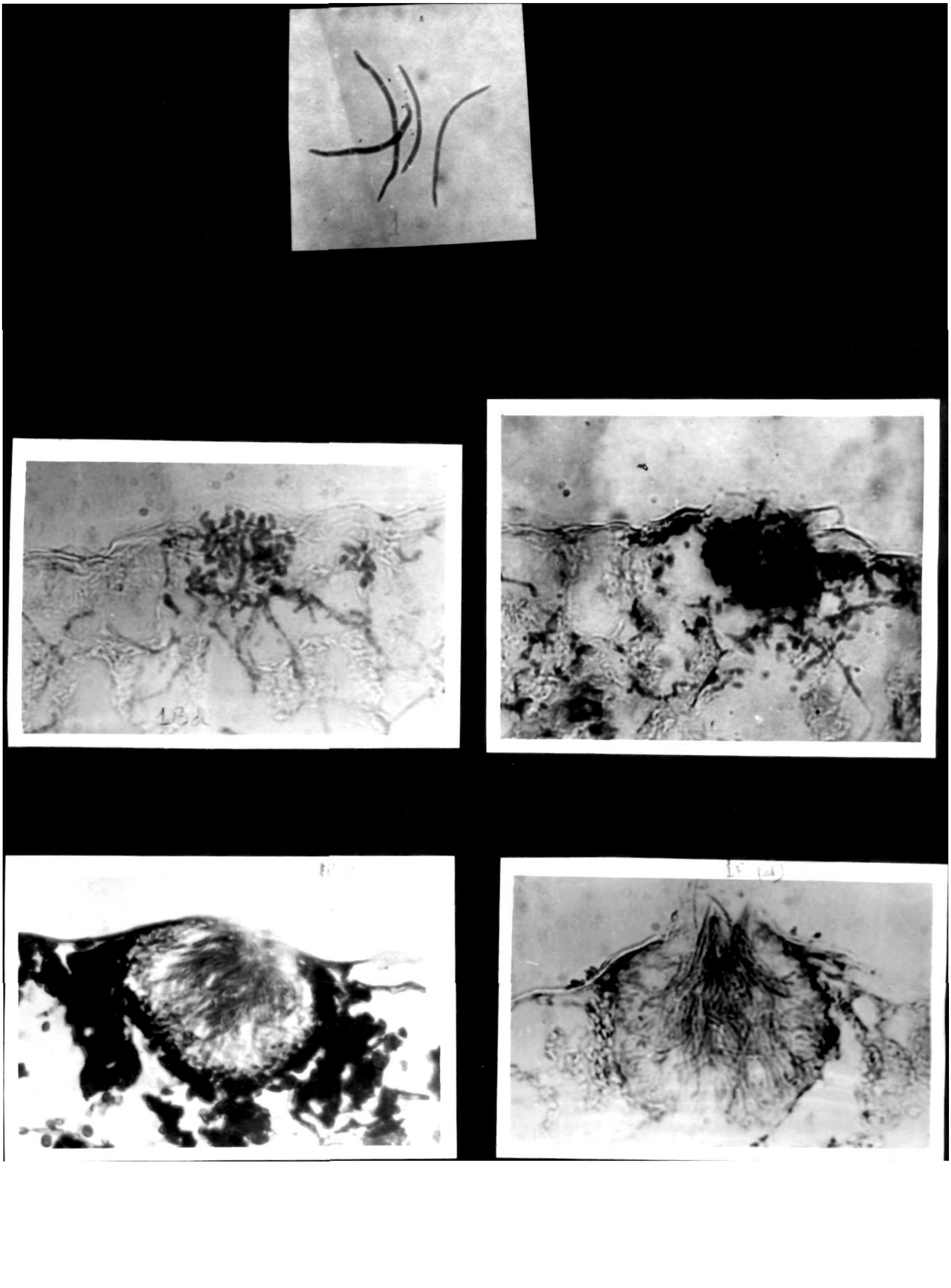
d) A well developed pycnidium with spores.

HY = Hyphae

Ep = Epidermis

Py = Pycnidium

Sp = Spores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characteristic of progressing, culture and measured 2.10-3.05 µm breadth with an average of 2.22 µm.

Conidia measured from 22.31-56.26 µm x 2.06-2.91 µm with an average of 38.42 x 2.17 µm.

From host:

Conidia measured from 21.34-62.11 µm x 2.06-2.91 µm and averaged to 41.46 x 2.11 µm. Number of Septa ranged from one to five and majority of them (39.8%) were 3 septate (plate 1). Pycnidia which were increased in the host tissue, measured from 77.5-130.0 µm x 72.5-147.5 µm with an average of 99.25 x 98.37 µm.

Symptomatology

Symptoms were observed on leaves petiole and stem, but not on pods. On leaves it first appears as small, circular water soaked spots (Plate 2a). These spots later become necrotic (Plate 2b). The margin of such necrotic spots will be of darkbrown in colour while the centre will of light brown to greyish in colour. Later in the centre of the spots black bodies appear, which are nothing but pycnidia (Plate 2c). Colour of the necrotic spots was more conspicuous on upper surface rather than on the lower surface. Further pycnidia were more commonly found on the upper surface than in lower surface. In severe cases, these spots coalase and produce blotches resulting in premature yellowing, drying and finally dropping of the leaf.

Plate 2. Different stages in the development of symptoms.

a) Appearance of water soaked spots.

b) Necrosis of water soaked spots. Note the absence of dark margins and the absence of pycnidia.

c) Final stage of symptoms. Note the dark margin limiting the spots and numerous pycnidia in the spots.


7c

Similar symptoms were observed on petiole and stem, but no pycnidia were observed. No symptoms were observed on pods in field or after artificial inoculations under glasshouse conditions.

Incubation period:

Incubation period ranged from 11 to 18 days. However in majority of the cases symptoms appeared after 14 days. The reasons for such a long incubation period are not known.

Spot size and number of pycnidia per spot:

One hundred spots which appeared to have not coalesed were measured using a scale with 0.5 mm divisions (Scale No. M1). The diameter of spot varied from 1.5 mm to 4.0 mm average to 2.74 mm. Number of pycnidia per spot also varied from 7 to 48.

Growth of the fungus on different solid media

This study was conducted to select a best medium for further studies on solid media. Eleven different media were used. The preparation of the media is described under "Material and Methods". Twenty ml of each medium was poured into a sterilised 3 inches petridish and replicated thrice. All the petridishes were inoculated uniformly with the fungus and incubated at 25 $\pm$ 1°C. After 30 days the radial growth of the colony was measured. Other characteristics

Table. I

Growth of *S. dolichi* on different solid media

Sl. No.	Media	Mean colony diameter (mm)
1.	Hawaker and Asthana agar	5.66
2.	Modified Cox's agar	9.00
3.	PDA	13.33
4.	Sabouraud dextrose agar	22.33
5.	Leonian agar	7.33
6.	Tochinai's agar	11.33
7.	Richard's agar	5.00
8.	Pod extract agar	4.33
9.	Leaf extract agar	13.00
10.	Seed extract agar	16.00
11.	Leaf + Seed + PDA	13.33

S.Em. ± 1.280

CD at 5% 2.67

4.33	5.00	5.66	7.33	9.00	11.33	13.00	13.33	13.33	16.00	22.33

Plate 3. Growth and cultural characteristics of *S. dolichi* on nine different solid media.

1. Seed extract agar
2. Modified Cox's medium
3. Potato dextrose agar
4. Sabouraud dextrose agar
5. Leonian agar
6. Tochinai's agar
7. Leaf + Seed + PDA
8. Leaf extract agar
9. Pod extract agar

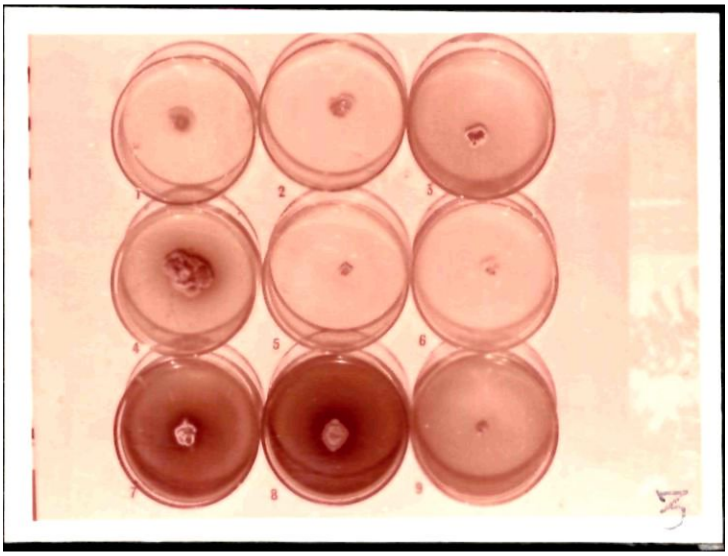


Table. II

Cultural characteristics of *S. dolichi* on different solid media.

Sl. No.	Medium	Colour of the colony	Margin and reaction with the medium	Growth and topography	Sporulation
1.	Asthana and Hawker's medium	Greyish white	Circular, dark	Poor, slightly raised	++
2.	Modified Cox's agar	Whitish	Wavy margin, brown	Medium, plain	++
3.	Potato dextrose agar	Greyish	Wavy margin, dark	Good, slightly raised	++++
4.	Sabouraud dextrose agar	Greyish black	Wavy margin, dark	Abundant, Very much raised	++++
5.	Leonian agar	Whitish	Wavy margin, brown	Poor, raised	++
6.	Tochinai'S agar	Greyish	Wavy margin, brown	Medium, plain	++
7.	Pod extract agar	Greyish white	Circular, dark	Poor, slightly raised	+
8.	Leaf extract agar	Greyish black	Circular, medium dark	Good, slightly raised	++++
9.	Seed extract agar	Whitish	Circular, white	Medium, plain	+++
10.	Seed + Leaf + PDA	Whitish with greyish centre	Wavy, dark	Medium, slightly raised	+++
11.	Richards' agar	Whitish	Circular, white	Poor plain	+++

Sporulation: + = Poor; ++ = Moderate; +++ = Good; ++++ = Excellent.

Figs. I and III. Effect of different media on growth of <u>Septoria</u> <u>dolichi</u>.

Fig. I. Solid Media

1. Richards agar
2. Leonian agar
3. Tochinai's agar
4. PDA
5. Seed extract agar
6. Sabouraud dextrose agar
7. Leaf + seed + PDA
8. Leaf extract
9. Modified Cox's agar
10. Asthana and Hawker's agar
11. Pod extract

Big. III. Liquid Media

1. Modified Cox's medium
2. Tochinai's medium
3. Leonian medium
4. Sabouraud dextrose medium
5. Seed extract
6. P.D. broth
7. Leaf extract
8. Richards medium
9. Asthana and Hawker's medium
10. Pod extract

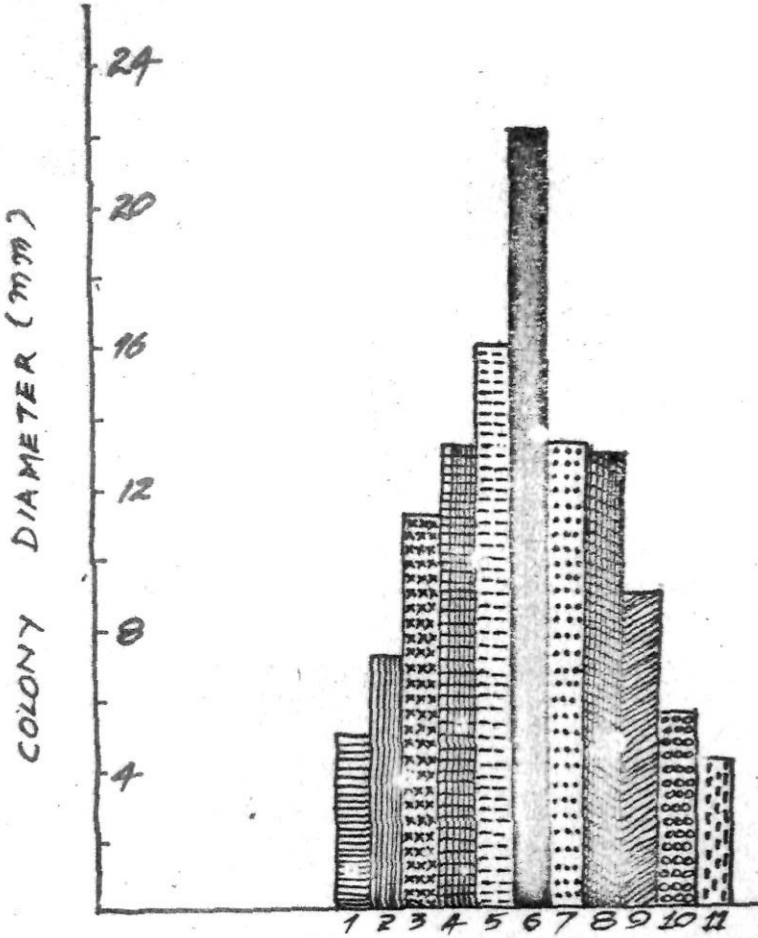


FIG I : SOLID MEDIA

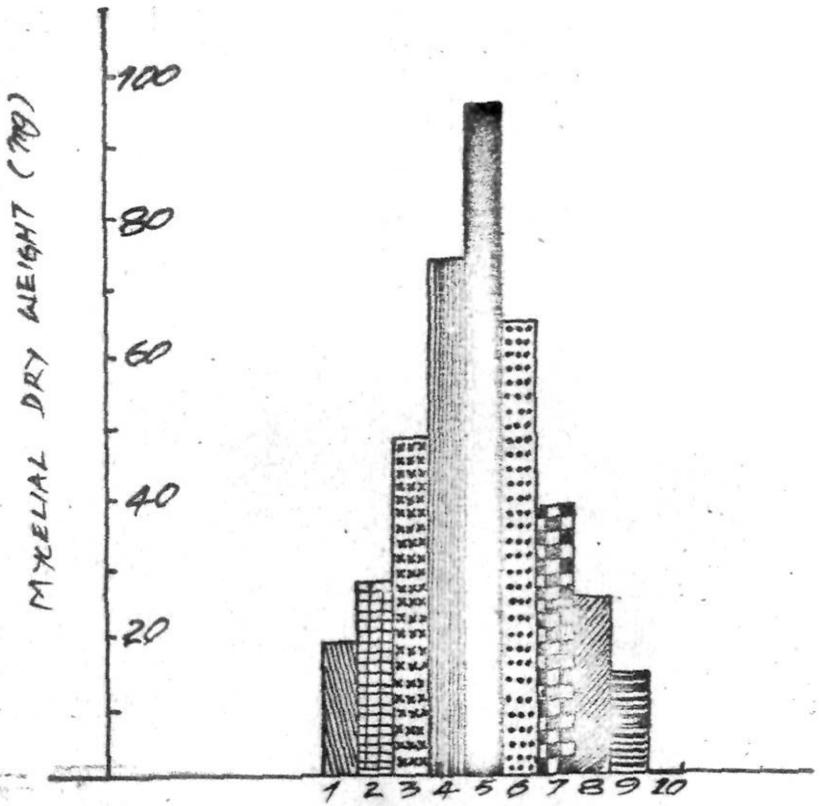


FIG. III LIQUID MEDIA

FIG. I & III EFFECT OF DIFFERENT MEDIA ON GROWTH OF *SEPTORIA DOLICHI*.

of the fungal colony such as reaction with the medium, margin of the colony, topography and extent of sporulation were also recorded. The results are presented in table I and II and Fig. I.

S. dolichi varied in growth on different media. Sabouraud dextrose agar supported maximum growth (22.33 mm) followed by seed extract agar (16.00 mm). There was significant difference between Sabouraud dextrose agar and the rest of media tried. Further there was no significant difference among seed extract agar, PDA and leaf + seed + PDA. Least amount of growth was recorded in pod extract (4.33 mm). The growth of S. dolichi on nine media is shown in Plate 3.

Growth phase of the fungus

Growth phase of the fungus was studied in potato dextrose broth. The first harvest of the mycelium was done on 8th day and subsequent harvests were done at 5 days intervals. The data obtained is presented in table III and Fig. II.

It can be seen that the growth of fungus increased upto 33 days and thereafter started declining. Hence the maximum incubation period for all the experiments was fixed as 30 days.

Growth of the fungus in different liquid media

In this study ten different liquid media were tried. The method of preparation is given under "Material and Methods". Twentyfive ml of each medium was used with three replications.

Table. III

Growth phase of S. *dolichi* in potato dextrose broth.

Sl. No.	Duration (days)	Dry mycelial weight (mgs)
1.	8	8.79
2.	13	12.82
3.	18	21.42
4.	23	27.68
5.	28	35.40
6.	33	37.00
7.	38	34.80
8.	43	30.48

Fig. II. Growth phase of *Septoria dolichi*.

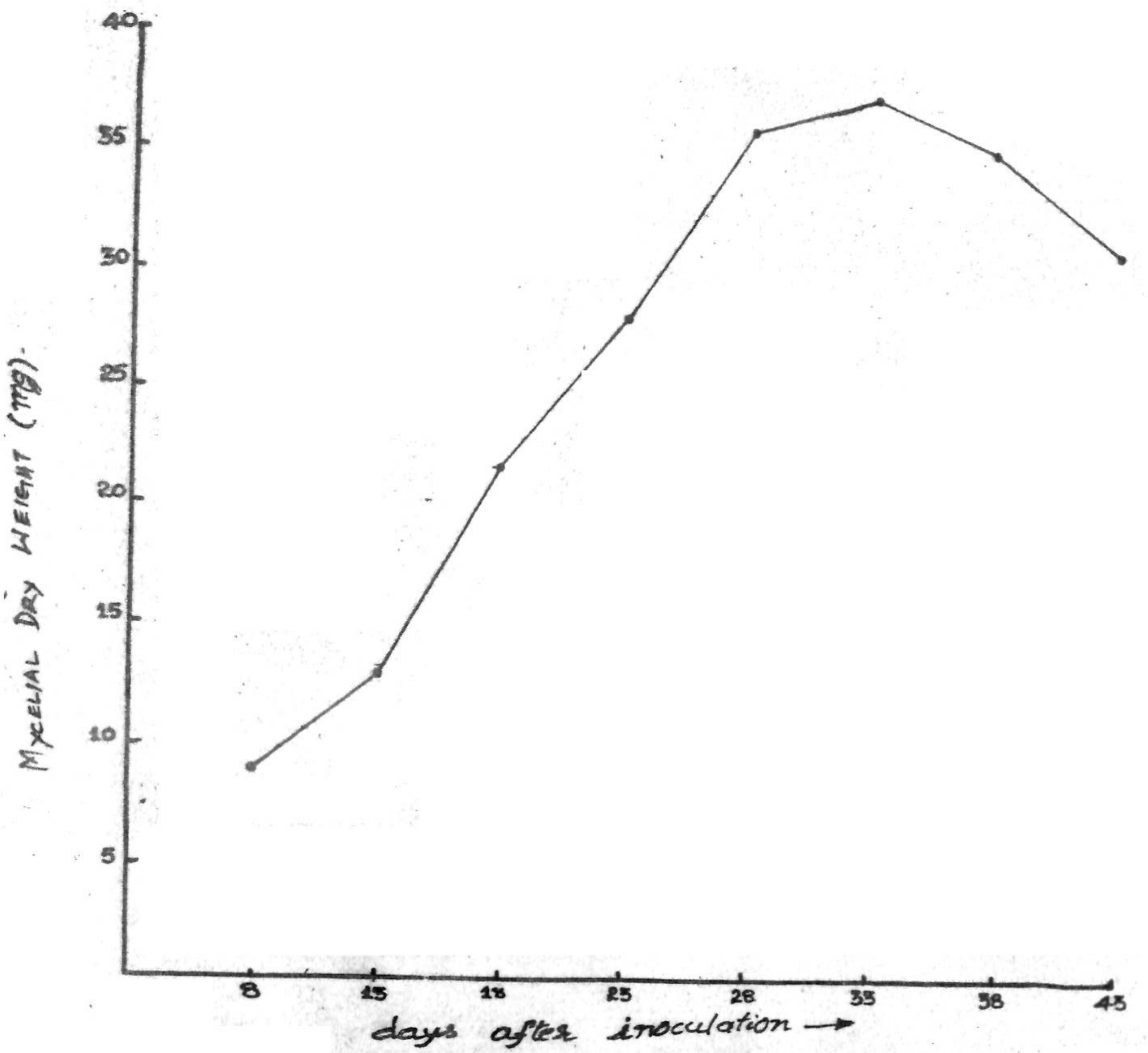


FIG III: Growth phase of *Septoria dolichi*

All the flasks were inoculated and incubated at 25 $\pm$ 1°C, after 30 days, the mycelial growth was harvested and dry mycelial weights were recorded. The data was analysed statistically and presented in table IV and fig.IIIV.

The extent of growth of the fungus was varying in different media. The dry mycelial weights differed significantly with maximum growth in seed extract followed by Sabouraud dextrose and potato dextrose broths. No growth was observed in pod extract. Growth on Richards' medium and Tochinai's medium did not differed significantly. Between Asthana and Hawker's medium and modified Cox's medium also, there was no significant difference. While all other media differed significantly.

Since there was no growth in pod extract, further study was made to know the factor inhibiting the growth of the fungus. The pods of Hebbal avare-3 (Ha-3) are known to secrete an oily substance which gives a peculiar smell to them. The pods taken from Ha-3 were kept in absolute alcohol for half an hour and later the pods were washed with tap water and used for preparing extraction. The extraction was also made from untreated pods. Twenty ml of seed extract, untreated pod extract and treated pod extract were transferred to 100 ml conical flasks separately and replicated five times. After sterilisation, all the flasks were inoculated and incubated at 25 $\pm$1°C. After 30 days the dry mycelial weights were recorded. The data obtained is presented in table V.

Table. IV

Growth of S. dolichi in different liquid media.

Sl. No.	Medium	Dry mycelial weight (mg)
1.	Asthana and Hawker's medium	15.33
2.	Modified Cox's medium	19.33
3.	Sabouraud dexture medium	73.66
4.	P.D. broth	64.66
5.	Seed extract	96.33
6.	Leaf extract	39.33
7.	Pod extract	nil
8.	Tochinai's medium	28.33
9.	Leonian medium	48.66
10.	Richards' medium	26.00

S.Em. ± 2.979

CD at 5% 6.27

0.00 $\overline{15.33 \quad 19.33}$ $\overline{26.00 \quad 28.33}$ 39.33 48.66 64.66 73.66 96.33

Table. V

Growth of *S. dolichi* in treated and untreated pods' extract.

Sl. No.	Medium	Mean mycelial dry weight (mg)	Sporulation
1.	Seed extract	65.3	nil
2.	Pod extract (alcohol treated)	58.2	Good
3.	Pod extract (un-treated)	nil	nil

There was no growth in the extract obtained from untreated pods and there was considerable growth of the fungus in the extract prepared from alcohol treated pods. Further good sporulation was observed in the extract of treated pods while there was no sporulation in seed extract at the end of 30th day.

Nutritional studies

Studies on utilization of different carbon sources:

The utilization of different carbon sources by S. dolichi was studied in Tochinai's medium. The results of the experiment are presented in table VI and fig. IV. The data was analysed statistically.

It can be seen that the fungus varied in utilization of different carbon sources of the ten carbon sources tried in this study viz., dextrin, glucose, glycerol, lactose, mannitol, rhamnose, sodium acetate, sodium citrate, soluble starch and sucrose, maximum growth was recorded on glucose followed by lactose, starch, sucrose and mannitol. Glycerol and rhamnose supported less growth while sodium acetate and sodium citrate supported no growth. However there was no significant difference among glucose, lactose, starch and sucrose.

Studies on utilization of different nitrogen sources:

The utilization of different nitrogen sources by S. dolichi was studied in Tochinai's medium. The results of the experiment are presented in table VII and fig. V.

Table. VI

Effect of different carbon sources on growth of *S. dolichi*.

Sl. No.	Carbon source	Mean mycelial dry weight (mg)
1.	Glucose	58.2
2.	Lactose	57.6
3.	Starch	56.8
4.	Sucrose	55.8
5.	Mannitol	52.2
6.	Dextrin	43.2
7.	Glycerol	38.6
8.	Rhamnose	33.4
9.	Sodium citrate	nil
10.	Sodium acetate	nil

S.Em. ± 2.267

CD at 5% 4.60

58.2	57.6	56.8	55.8	52.2	43.2	38.6	33.4	0.00	0.00

Fig. IV. Effect of carbon sources on the growth of *Septoria dolichi*.

1. Rhamnose
2. Glycerol
3. Mannitol
4. Starch
5. Glucose
6. Lactose
7. Sucrose
8. Dextrin
9. Sodium acetate
10. Sodium citrate

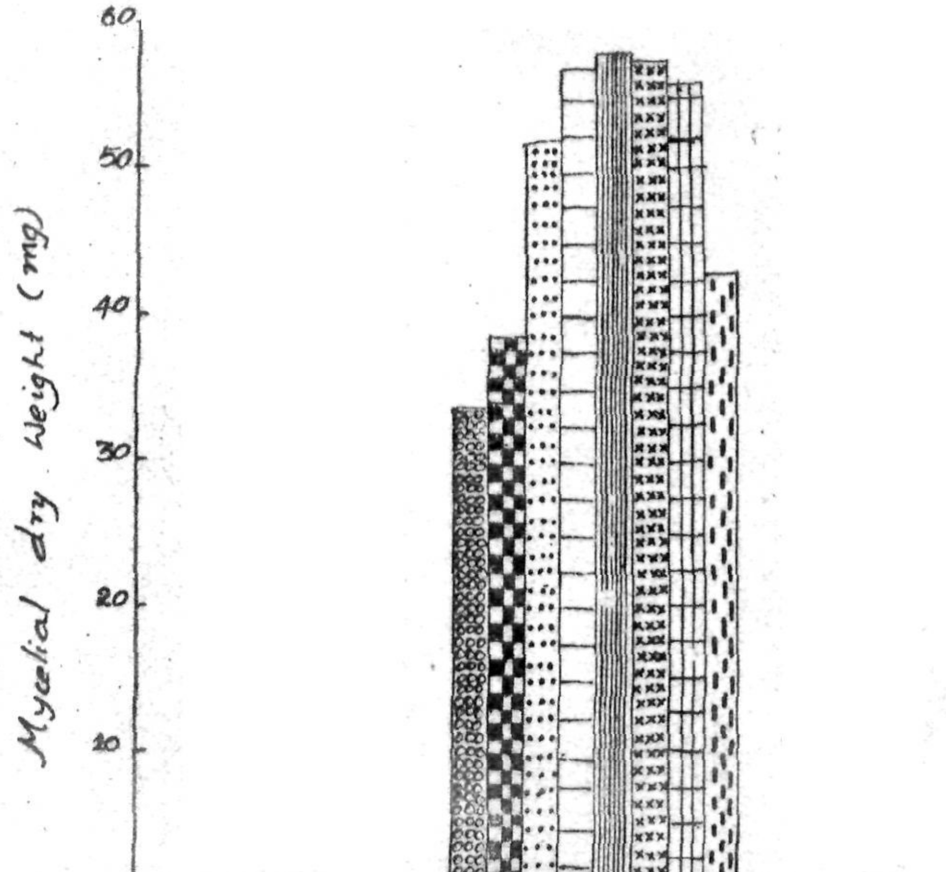


FIG. IV EFFECT OF CARBON SOURCES ON THE GROWTH OF SEPTORIA DOLICHI.

Table. VII

Effect of different nitrogen sources on growth of *S. dolichi*

Sl. No.	Nitrogen source	Mean mycelial dry weight (mg)
1.	Potassium nitrate	76.50
2.	Asparagine	70.25
3.	Lysine	62.75
4.	Proline	59.75
5.	Ammonium nitrate	53.75
6.	Ammonium sulphate	52.75
7.	Tryptophan	48.00
8.	Alanine	33.75
9.	Asparatic acid	nil
10.	Glutamic acid	nil

S.Em. ± 4.361

CD at 5% 8.94

0.00 0.00 33.75 48.00 52.75 53.75 59.75 62.75 70.25 76.50

Fig. V. Effect of nitrogen sources on the growth of *Septoria dolichi*.

1. Alanine
2. Tryptophan
3. Ammonium nitrate
4. Lysine
5. Potassium nitrate
6. Asparagine
7. Proline
8. Ammonium sulphate
9. Aspartic acid
10. Glutamic acid

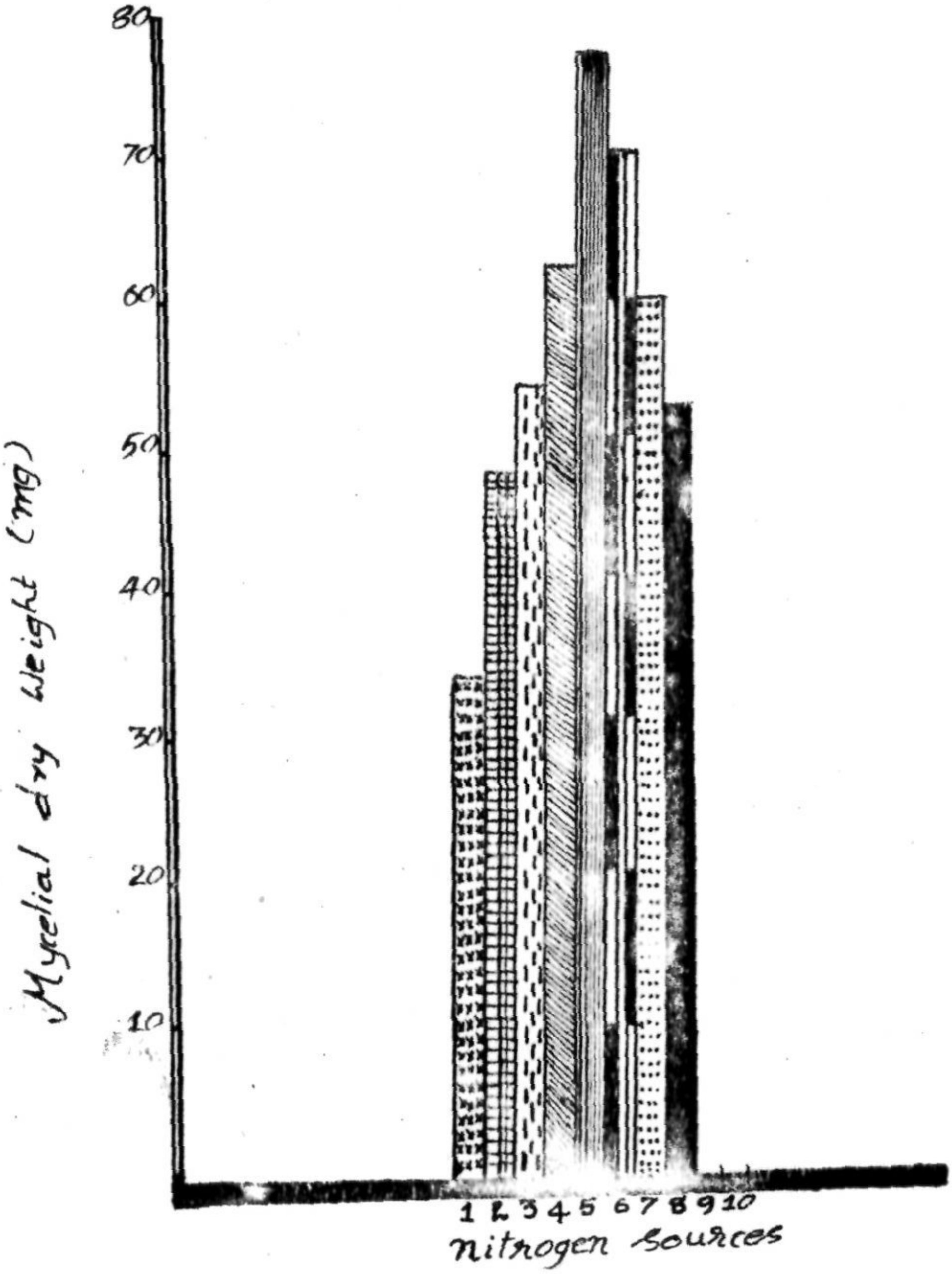


FIG. V EFFECT OF NITROGEN SOURCES ON THE GROWTH OF SEPTORIA DOLICHI.

The fungus showed a good deal of variation in utilizing different nitrogen sources. Among nitrogen compounds viz., aspartic acid, asparagine, ammonium nitrate, ammonium sulphate, alanine, glutamic acid, lysine, proline, potassium nitrate and tryptophan, used in this study, maximum growth was observed in the medium containing potassium nitrate as nitrogen source followed by asparagine and lysine. However there was no significant difference between potassium nitrate and aspergine. No growth was observed in the medium containing aspartic acid and glutamic acid as nitrogen sources.

<u>Physiological studies</u>

<u>Effect of pH on growth of the fungus:</u>

Growth of <u>S</u>. <u>dolichi</u> at 12 different levels of pH was studied, in Tochinai's medium. The detailed procedure has been explained under "Material and Methods". The pH levels used were 2.5, 3.0, 3.6, 4.1, 4.5, 5.1, 5.6, 5.9, 6.6, 6.9, 7.6 and 7.8(after sterilisation). All the flasks were inoculated with the fungus and incubated at 25 $\pm$ 1°C for 30 days. Afterwards dry mycelial weight and sporulation were recorded. The data was statistically analysed and is presented in table VIII and fig. VI.

The data shows that there was no growth at 2.6, 3.0 and 3.6. Further maximum growth was observed at 6.0 followed by 6.5 and 5.5. Least growth was observed at pH 4.0.

Table. VIII

Growth of S. dolichi at twelve different pH levels.

Sl. No.	pH levels (after sterilisation)	Mean dry mycelial weight (mgs)
1.	2.5	nil
2.	3.0	nil
3.	3.6	nil
4.	4.1	6.3
5.	4.5	12.6
6.	5.1	18.3
7.	5.6	30.0
8.	6.1	50.3
9.	6.6	44.6
10.	6.9	33.0
11.	7.6	25.3
12.	7.8	12.3

S.Em. ± 2.433

CD at 5% 5.16

6.3 12.3 12.6 18.3 25.3 30.0 33.0 44.6 50.3

Fig. VI. Effect of pH on the growth of S. *dolichi*.

1. 2.5
2. 3.0
3. 3.6
4. 4.1
5. 4.5
6. 5.1
7. 5.6
8. 6.1
9. 6.6
10. 6.9
11. 7.6
12. 7.8

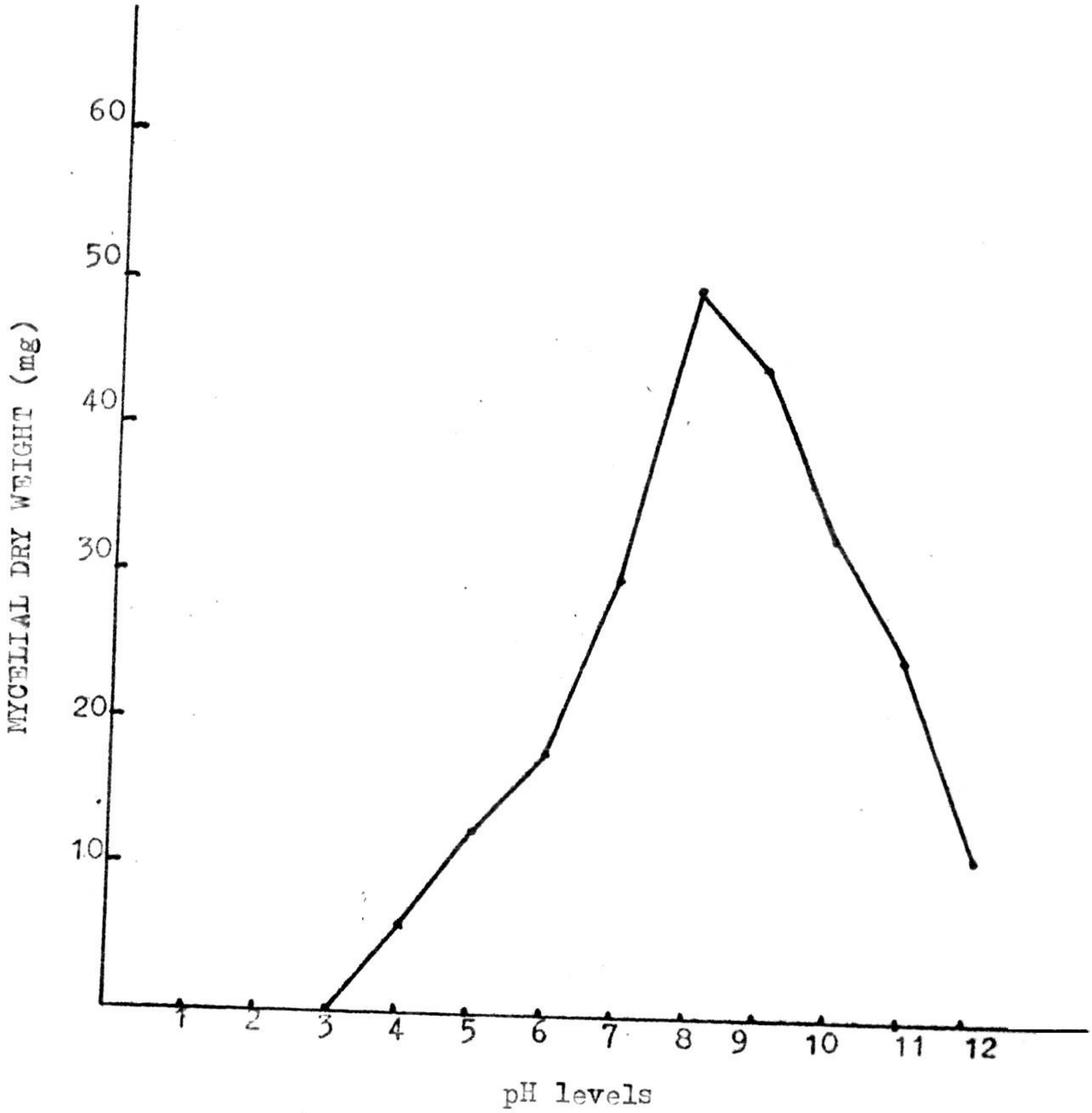


Fig. VI : EFFECT OF pH ON THE GROWTH OF *S. dolichi*.

Sporulation was observed only at pH 4.5 and 5.0 at the time of harvest. Further there was no significant difference between the dry mycelial weights at pH 7.8 and 4.5, 7.6 and 5.6, 5.6 and 6.9. The growth at pH at 6.1 and 6.6 differed significantly from the remining.

Growth of the fungus at different temperature:

The fungus was inoculated on to petridishes containing Sabouraud dextrose agar and incubated at 5, 10, 15, 20, 25, 30 and 35°C with four replications. After 25 days the colony diameter was measured and results are presented in table IX and fig. VII.

There was remarkable effect of temperature on growth and sporulation of the fungus. Maximum growth and sporulation was recorded at 25°C, followed by 20°C and 15°C. However, there was very poor growth and sporulation at 10°C and 30°C. Further, there was no growth at 5°C and 35°C. There was no significant difference between the growth at 20°C and 25°C. Further no significant difference was observed between growth at 20°C and 15°C. The growth of S. dolichi at different temperatures is shown in plate 4.

Effect of light on growth and sporulation:

Petriplates containing Sabouraud dextrose agar were inoculated with the fungus and were exposed to different treatments viz., continuous light, 12 hrs alternate cycle

Table. IX

Growth and sporulation of S. dolichi at seven different temperatures.

Sl. No.	Temperature(°C)	Mean colony diameter (mm)	Sporulation
1.	5	nil	-
2.	10	3.75	+
3.	15	10.00	++
4.	20	13.00	+++
5.	25	17.00	+++
6.	30	3.50	+
7.	35	0.00	-

S.Em. ± 8.51

CD at 5% 4.51

Sporulation: + poor
++ good
+++ excellent

17.00	13.00	10.00	3.75	3.50	0.00	0.00

Fig. VII. Effect of temperature on the growth of *S. dolichi*.

1. 5°C

2. 10°C

3. 15°C

4. 20°C

5. 25°C

6. 30°C

7. 35°C

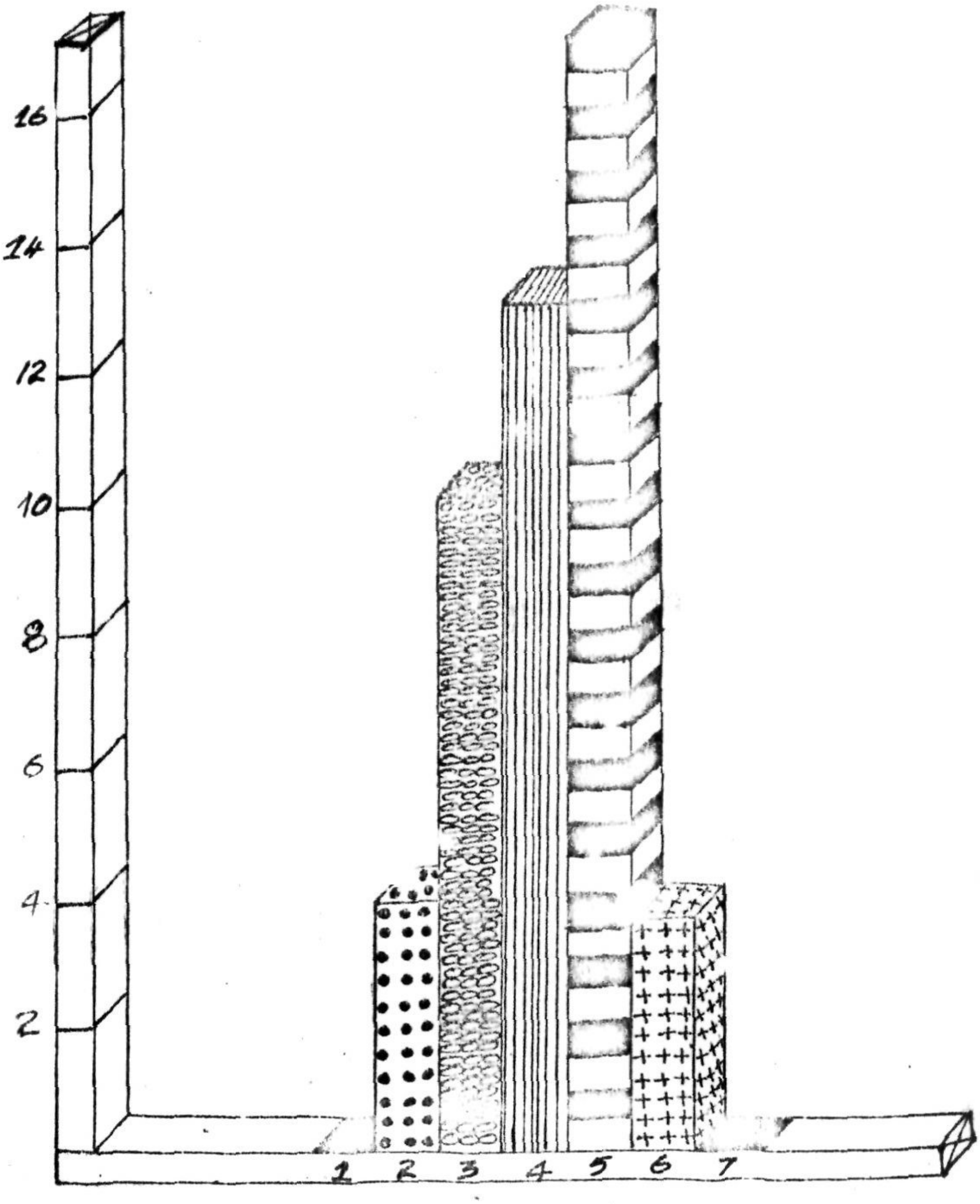


FIG IX EFFECT OF TEMPERATURE ON THE GROWTH OF S. DOLICHI.

Table. X

Effect of light on growth and sporulation of *S. dolichi*.

Treatments	Mean colony diameter (mm)	Growth characters	Sporulation
Continuous light	11.66	Dark greyish growth, raised in the centre.	Excellent
Continuous darkness	11.66	Whitish growth, not raised in the centre.	Poor
Alternate light and darkness	11.33	Dark greyish growth, slightly raised centre.	Good

Plate 4. Effect of temperature on the growth of *S. dolichi*.

a) 10°C

b) 15°C

c) 20°C

d) 25°C

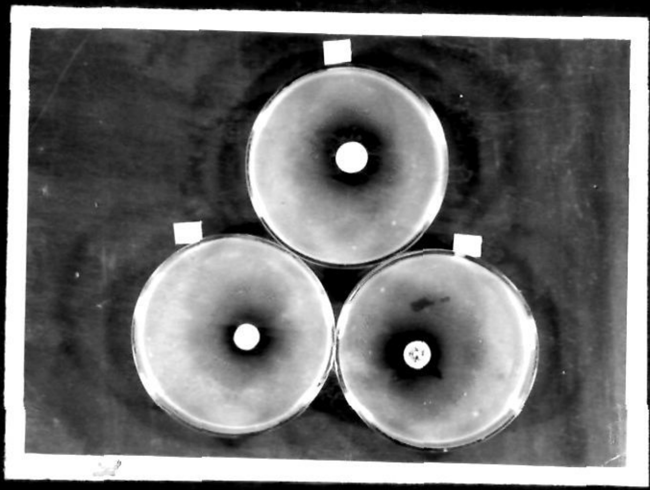
e) 30°C

Plate 5. Effect of light on the sporulation of *S. dolichi*.

1. Exposed to continuous light.

2. Exposed to alternate light and darkness.

3. Exposed to continuous darkness.



of light and dark and continuous darkness. After 20 days colony diameter was measured and sporulation was recorded. The results are presented in table X and plate 5.

There was no difference in colony diameter among the different treatments. However, there was significant effect on sporulation. Poor sporulation was observed in the petri-plates kept in complete darkness while excellent sporulation was observed in both the remaining treatments.

Spore germination studies

(a) Effect of different media on spore germination:

The spore germination in different media viz., host extract, glucose solution (1%), sucrose solution (1%), distilled water and tap water was studied. The percentage germination recorded in each medium after 24 and 48 hrs is presented in table XI and fig. VIII.

Maximum germination was noticed in host extract followed by glucose and sucrose solutions. Least germination was noticed in distilled water. In host extract 75.8 per cent and 86.6 per cent germination was recorded after 24 and 48 hrs respectively. While in distilled water only 5.8 per cent and 30.7 per cent germination was observed after 24 and 48 hrs, respectively.

Effect of temperature on spore germination:

Spore germination was studied in host extract at different temperatures viz., 0, 5, 10, 15, 20, 25, 30 and

Table. XI

Spore germination of *S. dolichi* in different media.

Sl. No.	Media	Germination percentage after	
		24 hrs.	48 hrs.
1.	Host extract	75.8	96.6
2.	Glucose solution (1%)	41.6	81.8
3.	Sucrose solution (1%)	20.6	68.0
4.	Tap water	12.9	62.2
5.	Distilled water	5.8	30.7

Fig. VIII. Effect of different media in spore germination of *Septoria dolichi*.

1. Host extract
2. Glucose solution (1%)
3. Sucrose solution (1%)
4. Tap water
5. Distilled water

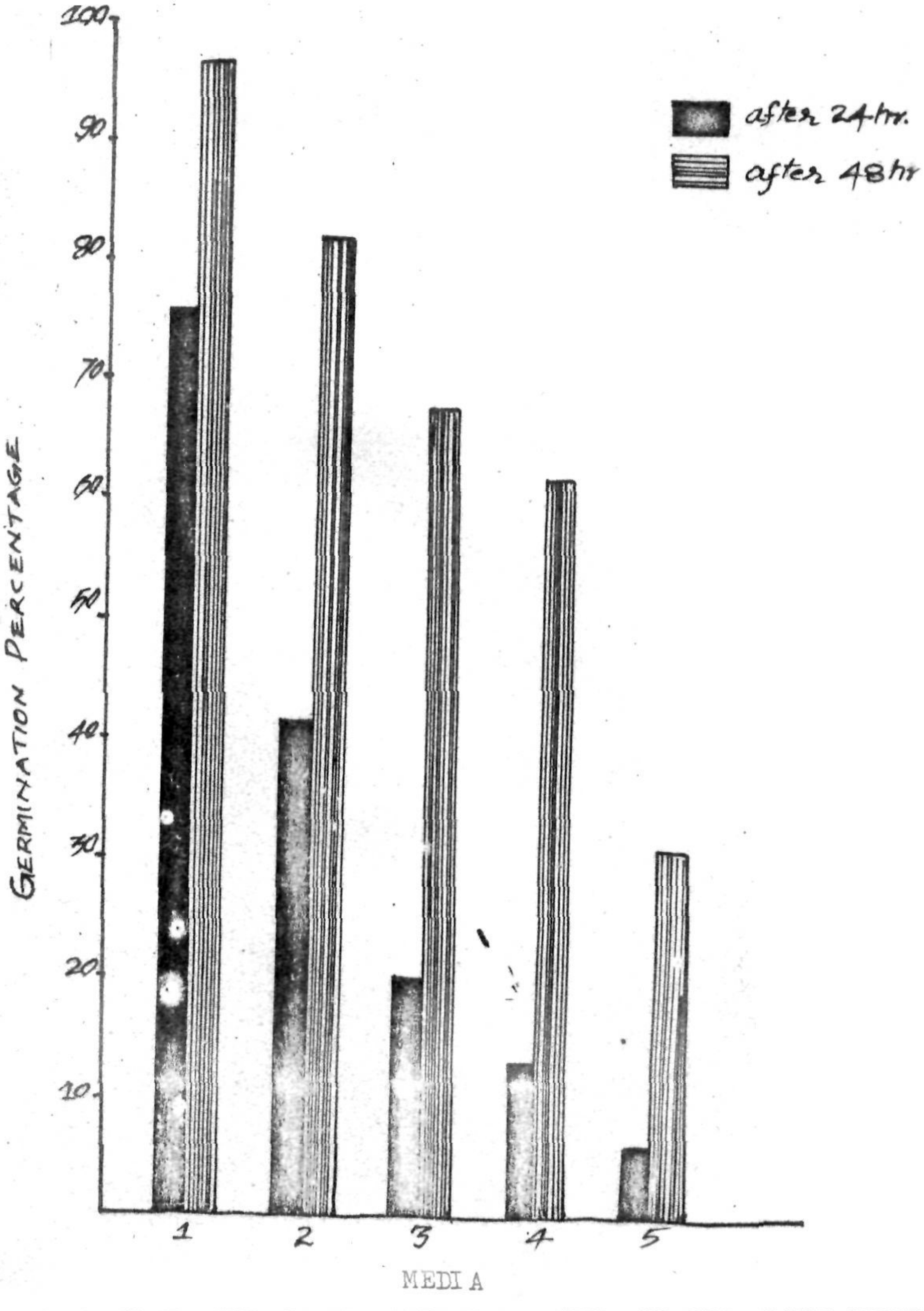


FIG. VIII EFFECT OF DIFFERENT MEDIA ON THE SPORE GERMINATION OF SEPTORIAA DOLICHI.

Table. XII

Spore germination of _S. dolichi_ at different temperatures.

Sl. No.	Temperature (°C)	Per cent germination (after 48 hrs.)
1.	0	nil
2.	5	nil
3.	10	9.60
4.	15	24.50
5.	20	78.63
6.	25	92.80
7.	30	32.50
8.	35	7.20

Fig. IX. Effect of temperature on spore germination of *Septoria dolichi*.

1. 0°C
2. 5°C
3. 10°C
4. 15°C
5. 20°C
6. 25°C
7. 30°C
8. 35°C

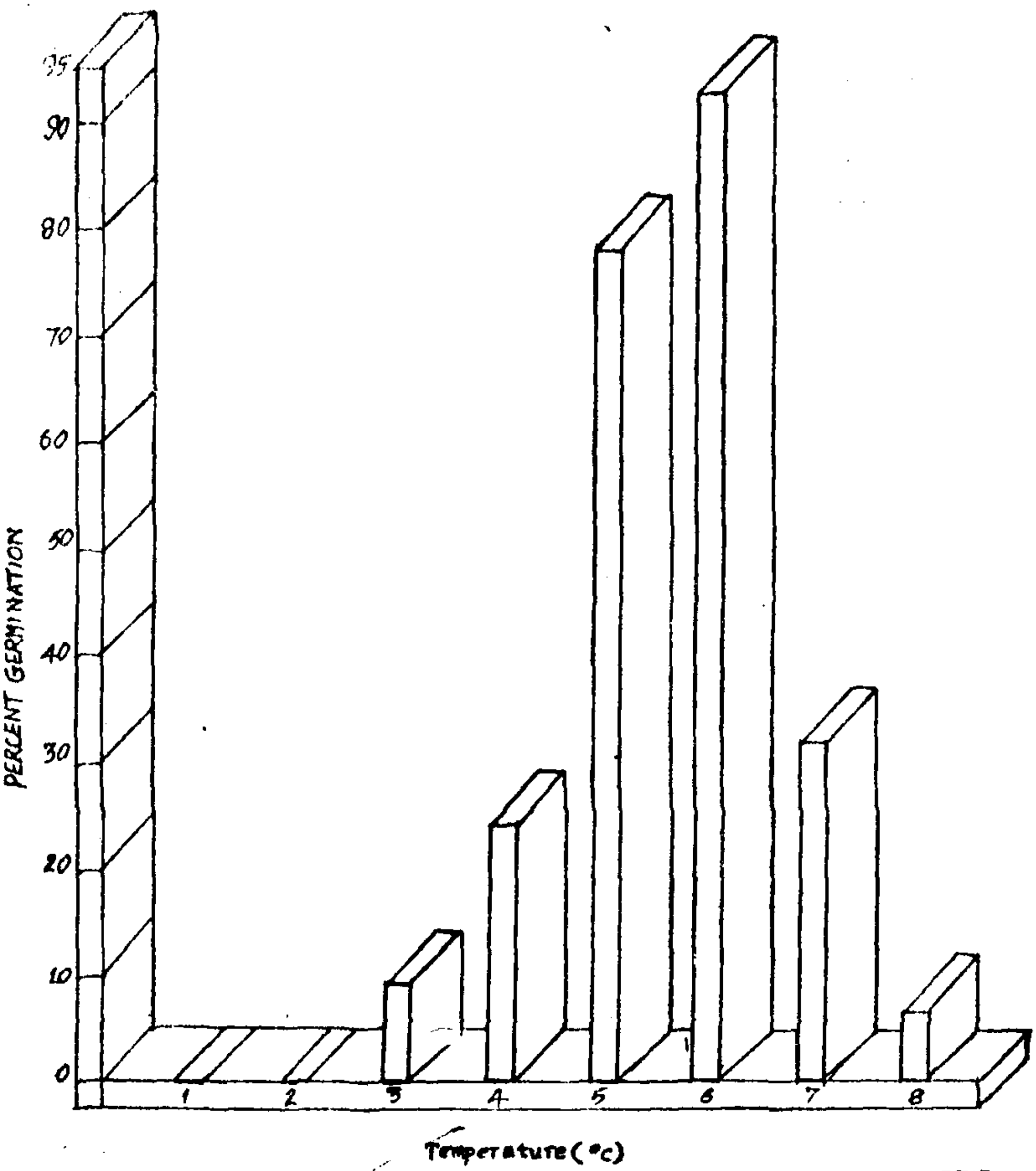


FIG. IX : EFFECT OF TEMPERATURE ON SPORE GERMINATION OF SEPTORIA DOLICHI.

35°C. The percentage germination recorded after 48 hrs at different temperatures is presented in table XII and fig. IX.

Maximum germination was noticed at 25°C followed by 20°C and 30°C. No germination was observed at 0°C and 5°C. At 35°C slight germination was observed.

<u>Effect of relative humidity on spore germination</u>:

Spore germination was studied at different RH levels artificially maintained in closed containers using saturated salt solutions. A drop of spore suspension was placed on a coverslip and allowed to dry. After which they were transferred to different RH levels. Percentage germination was recorded after 48, 72, 96 and 120 hrs. The results are presented in table XIII.

From the table XIII it can be seen that spore germination was maximum at 100 per cent RH, after 48 hrs, slight amount of germination (2.3%) was observed at 98 per cent RH with no germination below 98 per cent RH.

However, at the end of 120 hrs, considerable amount of germination (24.24%) was observed at 98 per cent RH while at 96 per cent only 9.33 per cent spores showed germination. Germination and even swelling of spores were not observed at RH of 92 per cent and below.

Table. XIII

Spore germination of S. dolichi at different levels of relative humidity.

Relative humidity(%)	Per cent germination after				Remarks
	48 hrs.	72 hrs.	92 hrs.	120 hrs.	
100	92.0	-	-	-	Germination counts were stopped because of crowded growth of hyphae.
98	2.3	6.80	14.16	24.24	Short germ tubes and several ungerminated but swelled spores were observed.
96	-	4.25	7.76	9.33	-do-
92	-	-	-	-	Swelling of spores not observed.
88.5	-	-	-	-	-do-
85.0	-	-	-	-	-do-
75.5	-	-	-	-	-do-

Spore germination on pods:

Since no pod infection was observed, spore germination studies were conducted on pods also. A loop full of spore suspension was placed within a circle previously marked on the pods nd leaves and was allowed to dry. Then the whole plant was transferred to a humid chamber. After 48 hrs the pods and leaves were removed. The earlier marked circles were cut and stained with lactophenol. Then the bits were mounted in lactophenol and observed directly using a light microscope. The per cent germination was calculated, the percentage germination on leaf was used as control.

After 48 hrs maximum germination (82%) was noticed on leaf, while on pods only 10 per cent germination was observed. When the pods were removed after 5 days and observed, 78 per cent germination was noticed.

Disease development

In this experiment, D. lablab variety, Hebbal avare-3 was sown in 3 x 3 m^2 plots at an interval of 15 days. First sowing was done on 26.8.1977 and subsequent sowings were done on 9.9.1977, 24.9.1977, 9.10.1977 and 24.10.1977.

The observations were recorded in each plot as described under "Material and Methods". Afterwards percentage number of leaves infected per plant was calculated by taking, number of diseased leaves per plot and total number of leaves/plant into consideration.

Table. XIV

Incidence of disease at different intervals of time after sowing.

Sl. No.	Date of sowing	Percentage No. of leaves infected/plant after				
		15 days	30 days	45 days	60 days	75 days
1.	26.8.1977	-	-	2.84	25.79	55.20
2.	9.9.1977	-	12.80	36.90	46.10	81.50
3.	24.9.1977	-	34.97	43.19	56.80	78.40
4.	9.10.1977	3.20	14.69	36.30	25.80	19.50
5.	24.10.1977	12.49	30.30	20.60	10.70	8.30

Table. XV

Severity of disease at different intervals of time after sowing.

Sl. No.	Date of sowing	Percentage leaf area affected after				
		15 days	30 days	45 days	60 days	75 days
1.	26.8.1977	-	-	0.02	0.25	5.40
2.	9.9.1977	-	0.03	0.69	1.35	6.76
3.	24.9.1977	-	0.31	1.33	9.90	12.80
4.	9.10.1977	0.02	1.05	3.35	2.03	1.90
5.	24.10.1977	2.10	2.50	1.83	0.90	0.65

Table. XVI

Weather date for the period from 26.8.1977 to 7.1.1978.*

Sl. No.	Dates	Mean temperature (°C)	Mean RH (%)	Mean Rainfall (mm)	No. of rainy days
1.	26.8.77 - 30.8.77	23.55	77.8	0.78	1
2.	31.8.77 - 4.9.77	24.18	74.1	0.04	1
3.	5.9.77 9.9.77	24.70	60.4	0.02	1
4.	10.9.72 - 14.9.77	24.30	69.8	0.84	2
5.	15.9.77 - 19.9.77	24.80	74.4	10.20	1
6.	20.9.77 - 24.9.77	24.37	79.4	4.36	3
7.	25.9.77 - 29.9.77	24.70	78.8	6.58	3
8.	30.9.77 - 4.10.77	24.50	78.3	-	-
9.	5.10.77 - 9.10.77	24.05	79.4	23.00	3
10.	10.10.77 -14.10.77	23.54	76.7	3.88	2
11.	15.10.77 -19.10.77	23.72	85.0	5.98	5
12.	20.10.77 -24.10.77	22.53	84.5	0.80	2
13.	25.10.77 -29.10.77	21.68	78.6	9.36	3
14.	30.10.77- 3.11.77	22.94	72.0	-	-
15.	4.11.77 - 8.11.77	23.24	78.5	11.24	2
16.	9.11.77 -13.11.77	21.61	79.6	3.30	1
17.	14.11.77 -18.11.77	21.52	82.6	0.68	3
18.	19.11.77 -23.11.77	22.40	77.2	2.08	1
19.	24.11.77 -28.11.77	22.41	88.0	4.20	5
20.	29.11.77 - 3.12.77	22.51	78.0	0.14	1
21.	4.12.77 - 8.12.77	21.27	68.0	-	-
22.	9.12.77 -13.12.77	20.22	67.0	-	-
23.	14.12.77 -18.12.77	18.88	66.8	-	-
24.	19.12.77 -23.12.77	20.34	68.1	-	-
25.	24.12.77 -28.12.77	19.69	64.3	-	-
26.	29.12.77 - 2.1.78	21.59	65.5	-	-
27.	3.1.78 - 7.1.78	21.35	67.9	-	-

* Each figure is the average of 5 values.

Percentage area affected per leaf was calculated by averaging the disease intensity of three leaflets. By considering the entire leaves per plant as 100, percentage leaf area killed by the pathogen per plant was calculated.

The meteorological data was obtained from M.R.S., Hebbal, Bangalore and presented in table XVI. The results of the experiment are presented in tables XIV and XV and figs. X and XI.

From the figures, it can be seen that the disease in the first sown crop was observed only after 45 days whereas in second and third sown crops it appeared within 30 days. In case of fourth and fifth sowings it appeared within 15 days only. This might be due to low relative humidity (less than 75%) during first 25 days with less amount of precipitation. Further, mean temperature was more than 24°C for nearly first 45 days. Maximum amount of disease was noticed in the second sown and crop followed by third sown crop. In all the first three crops disease increased with time. However, in case of fourth and fifth sowings, disease peak reached in 45 and 30 days, respectively and thereafter it started declining. This might be partly due to increase in number of leaves since the plants were still in active growth stage. From the fig. X, it is clear that 100 days after 23.11.1977 there was quick decline in RH and precipitation was almost negligible. Further, mean temperature was below 21.27°C.

From the data it can be inferred that for the infection of S. dolichi high relative humidity with frequent rains and

Fig. X. Effect of weather on disease incidence.

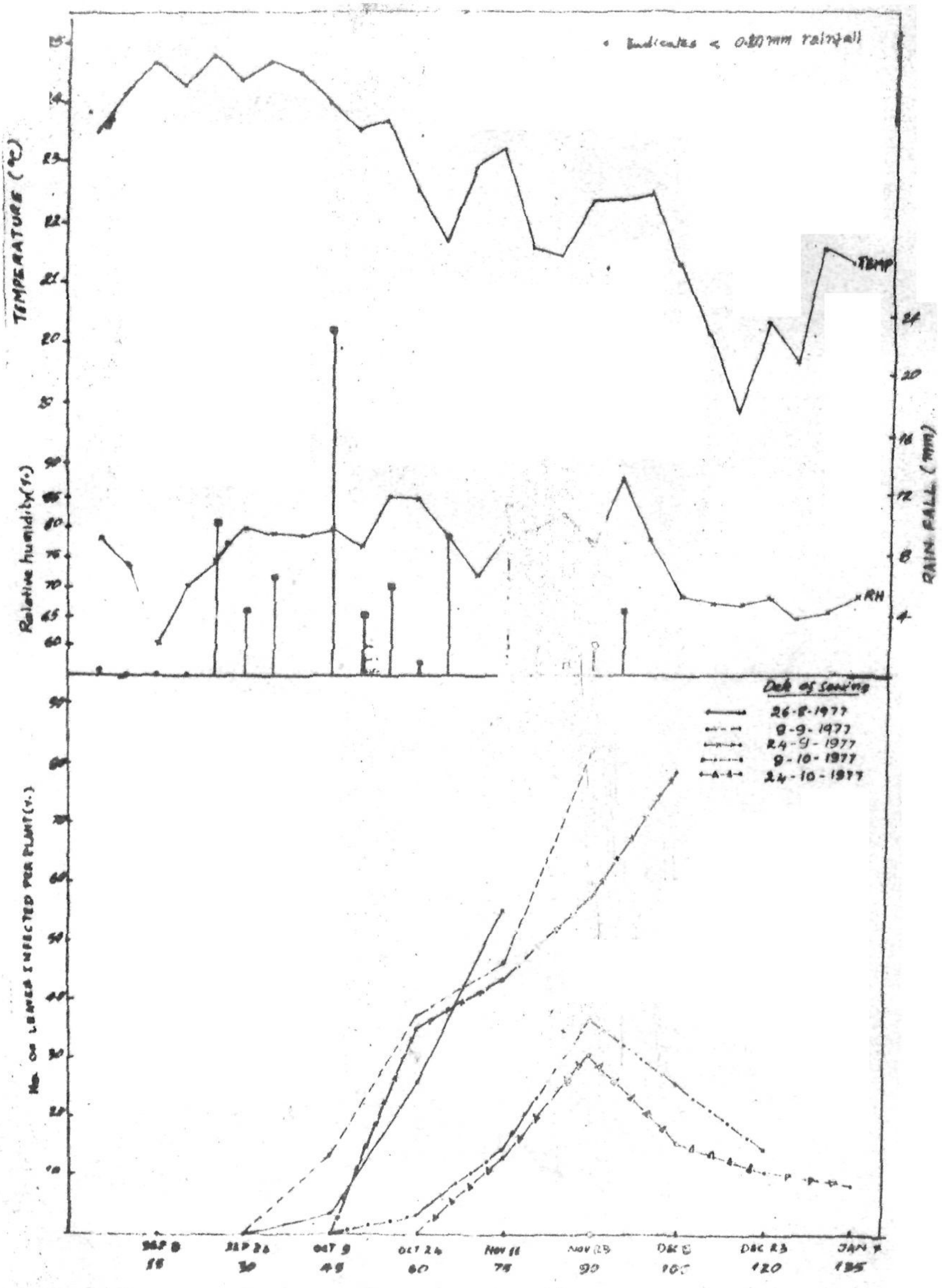


FIG. X EFFECT OF WEATHER ON DISEASE INCIDENCE.

Fig. XI. Effect of weather on disease severity

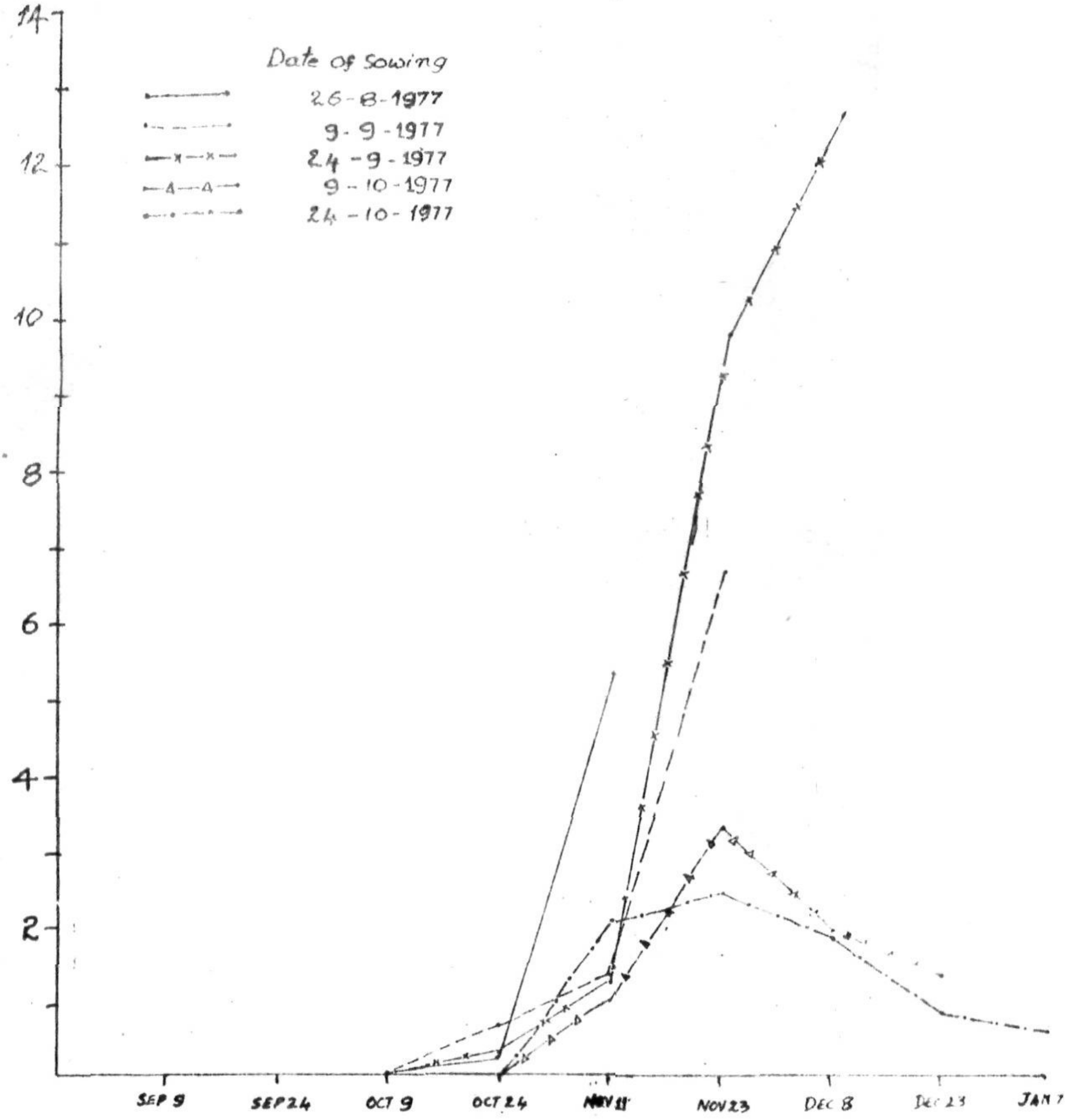


FIG. XI EFFECT OF WEATHER ON DISEASE SEVERITY.

low temperature are needed. However for the quick spread of the disease frequent rains are a must, since the spores in the pycnidia are easily disseminated by rain drops in a windy weather.

The disease intensity or leaf area damaged also increased with the age of the crop. Maximum severity of the disease was noticed in the crop sown on 24.9.1977 (12.80%) followed by the crops sown on 9.9.1977 (6.76%) and 26.8.1977 (5.4%). However the disease intensity increased during first 45 days and 30 days in the 4th and 5th sown crops, respectively and later started declining. Lowest intensity was noticed in the 5th sown crop followed by 4th sown crop. Thus it appears the crop sown particularly during September is attacked by S. dolichi to a severe extent. The low intensity of disease in 4th and 5th sown crops was mainly due to decrease in infection and increase in the foliage since the crops were still in actively growing state when the rain which is essential for spreading of the disease had stopped.

Effect of duration of 100 per cent of relative humidity on infection

This study was conducted as detailed under "Material and Methods" symptoms were observed only in plants exposed to high relative humidity (near 100%) for 12 hrs and more.

Mode of penetration of the fungus

Mode of penetration of host by S. dolichi was studied by following the method of Diener (1955). Germ tubes were observed entering through stomata. However, no direct penetration was observed. Further many germ tubes passing over stomata without penetrating them, were also observed.

Histopathology and histochemistry of diseased leaves

These studies were made as described under "Material and Methods".

Anatomy of healthy leaf:

The healthy leaf showed dorsiventral differentiation. The upper part of the leaf consisted of a single layer of epidermal cells which were rectangular in outline and lacked chloroplasts. This layer was covered by a thin cuticle. Below this layer, one to 2 layers of palisade parenchyma was present, consisting of columnar cells with many discoid chloroplasts. Below the palisade layer, spongy parenchyma was present made up of isodimatrical cells with less number of chloroplasts. The lowest part of the leaf was bounded by lower epidermis which also lacked chloroplasts. Cells which were parallel to the surface of the leaf were also observed near the vascular tissues (plate 8a).

Thickness of the leaf:

Thickness of the healthy leaf averaged to 169 µm, whereas that of stage-I was 121.25 µm and of stage-II was 94.5 µm.

Plate 6. Photomicrographs of leaf sections tested for total proteins by MBPB method.

a) Healthy leaf showing protein positive bodies in both the palisade and spongy cells.

b) Diseased leaf at stage-I showing increase in concentration of proteins in palisade cells while spongy cells showing decreased concentration.

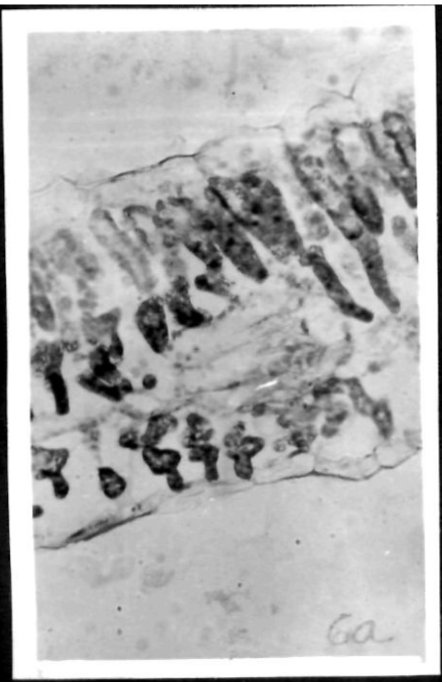
c) Diseased leaf at stage-II. Note that all the protein positive bodies in the entire mesophyll tissue have disappeared. Fungal structures are protein positive.

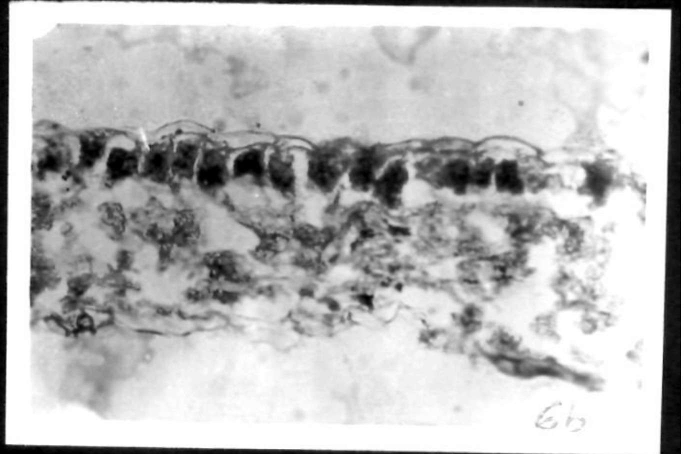
Ep = Epidermis

Pa = Palisade parenchyama

Sp = Spongy parenchyama

Py = Pycnidium


6a


6b

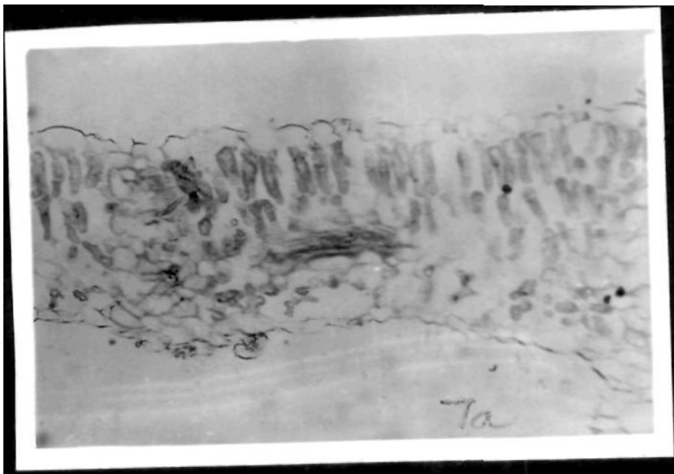

6c

Plate 7. Photomicrographs of leaf sections tested for total RNA content with Azur-B method.

a) Healthy leaf. Palisade and spongy cells showing almost equal distribution of RNA.

b) Diseased leaf at stage-I. Showing apparent increase in RNA concentration in palisade cells and decrease in spongy cells.

c) Diseased leaf at stage-II, showing complete loss of RNA in the mesophyll tissue. Hyphae (Hy) are rich in RNA.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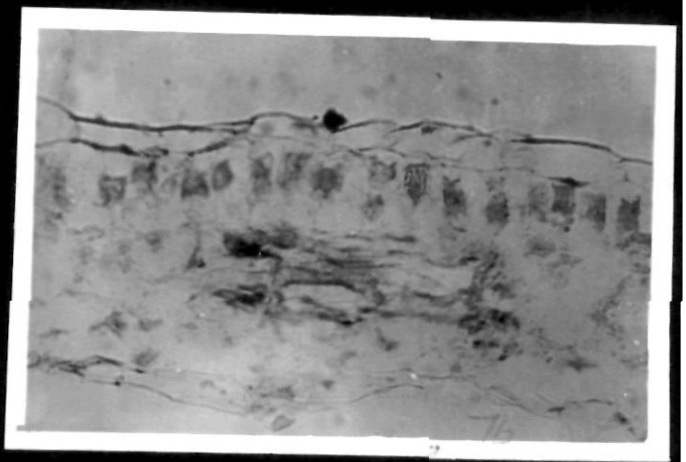
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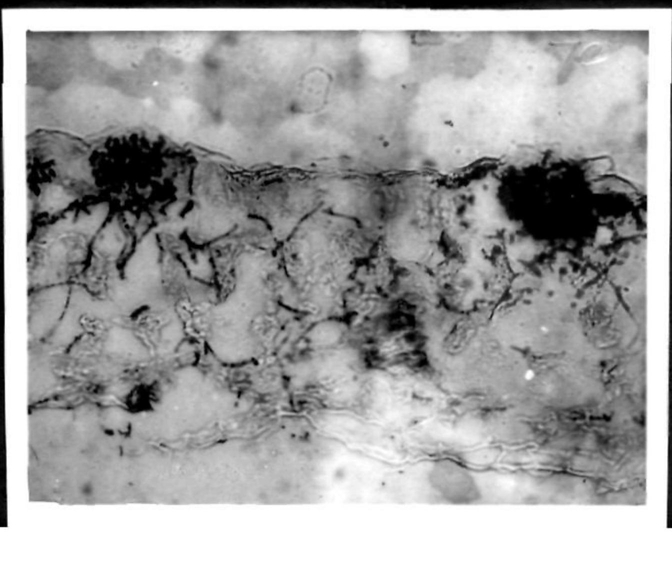

7c

Changes in protein content:

The healthy leaf tissue stained for total proteins with MBPB showed positive reaction for proteins only in palisade and spongy tissues (Plate 6a). Shrunken palisade cells showed an increase in the proteins, while spongy tissues showed signs of degradation in the leaf tissue at stage-I (Plate 6b). However, in the leaf tissue at stage-II, all the protein positive bodies in the entire mesophyll disappeared. Only spores were protein positive (Plate 6c).

Changes in RNA content:

Both the palisade and spongy cells of healthy tissue showed almost equal distribution of RNA (Plate 7a). The palisade cells of leaf tissue at stage-I showed increased RNA while spongy cells showed decreased RNA (Plate 7b). Further in the tissue at stage-II, the entire mesophyll showed complete loss of RNA. Fungal hyphae and spores were Azur B positive indicating the presence of rich RNA (Plate 7c).

Changes in polysaccharide content:

In healthy leaf both palisade and spongy cells showed polysaccharide grains in their plastids. The cells surrounding the vascular tissues also had small polysaccharide grains (Plate 8a). In the tissue at stage-I, clumping of polysaccharide grains was seen both in palisade and spongy

Plate 8. Photomicrographs of leaf sections tested for total insoluble polysaccharides with PAS.

a) Healthy leaf showing polysaccharide grains in palisade and spongy cells as well as in the cells surrounding the vascular tissue (V).

b) Diseased leaf at stage-I showing clumping of polysaccharide grains in both palisade and spongy cells.

c) Diseased leaf at stage-II showing the signs of degradation of polysaccharide grains making them sparse in the tissue.

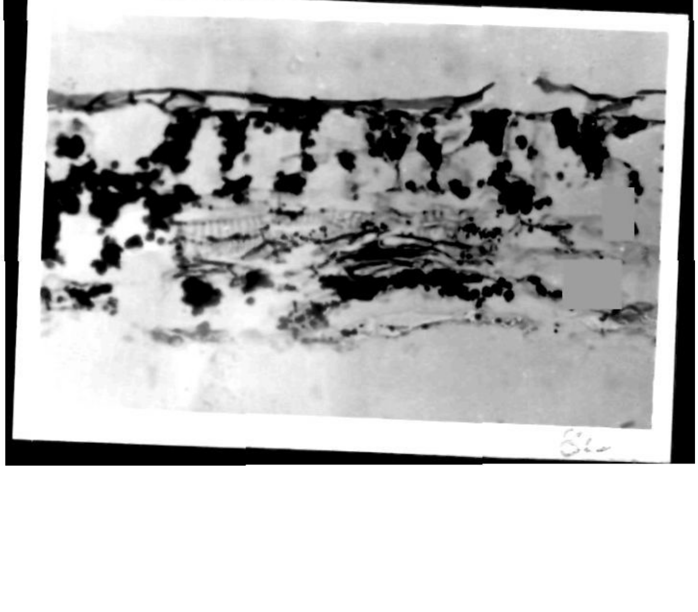
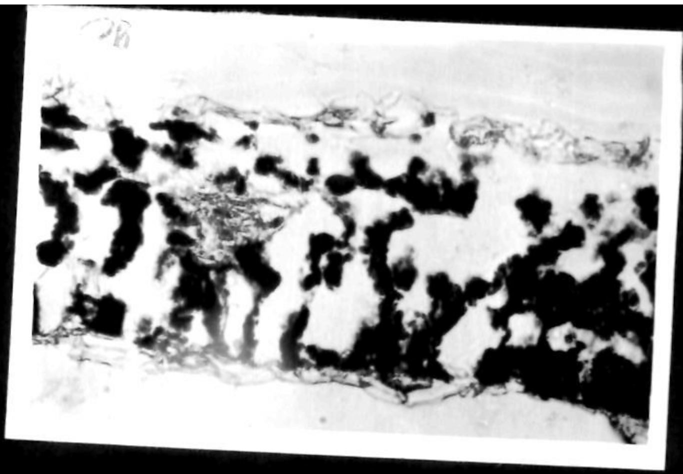


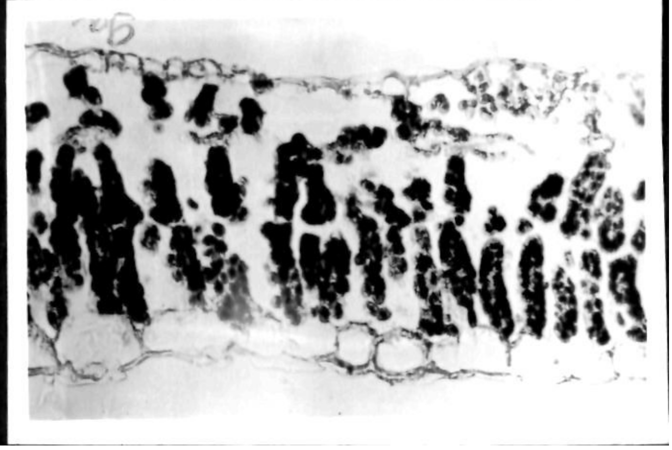
Plate 9. Photomicrographs of leaf sections tested for starch with I-KI.

a) Healthy leaf showing PAS positive polysaccharide grains are starch grains.

b) Diseased leaf at stage-I showing the clumping of the starch grains.

c) Diseased leaf at stage-II showing the signs of degradation of starch grains.




9Б

cells (Plate 8b). Reduction in the size of clump, making the tissue almost clear was observed in the tissue at stage-II. The hyphae and spores showed rich polysaccharide content (Plate 8c).

Changes in starch content:

The PAS positive grains in previous experiment were found to be starch. Clumping of starch grains and their reduction in the content of starch was observed (Plates 9a, 9b and 9c).

Biochemical changes in the host after infection

With a view to study the biochemical changes that would occur in the leaves as a result of infection, the following studies were made and the results are presented in table XVI.

Changes in phenol content:

Total phenols and ortho-di-hydroxy phenols in the diseased and healthy leaves were estimated by the method of Bray and Thorpe (1954) and Johnson and Schaal (1952), respectively. It was observed that healthy leaf contained 0.530 mg of total phenols/gm of leaf tissue (fresh) while diseased leaf contained 0.975 mg/g of leaf tissue. The diseased leaf contained 0.363 mg of OD phenols/gm of leaf tissue as against 0.249 mg/g of healthy leaf tissue. Thus it can be seen that both total phenols and OD phenols increased in the diseased leaves compared to healthy leaves.

Table. XVII

Biochemical changes in the leaves of *D. lablab* infected with *S. dolichi*.

Sl. No.	Constituent	Quantity in mg/g of leaf (fresh)		Increase or decrease over healthy(%)
		Healthy	Diseased	
1.	Total phenols	0.530	0.975	+ 83.9
2.	OD phenols	0.249	0.363	+ 45.7
3.	Amino nitrogen	0.450	0.832	+ 84.8

Changes in amino nitrogen:

Amino nitrogen was estimated by the method of Spies (1957). The results, obtained indicate that there was an increase in amino nitrogen content in diseased leaves as compared to healthy leaves.

Host range studies:

Among the ten plant species tested (mentioned in Material and methods) for pathogenicity, none of the plants showed infection. Thus it appears that *S. dolichi* cannot infect plants other than *Dolichos lablab* and the pathogen is host specific.

Perpetuation of the fungus

The diseased leaves were collected and stored in paper bags under laboratory conditions. At fortnightly intervals the spore germination test was conducted to know the viability of the pathogen. The spores did not germinate after 240 days (8 months).

Similarly the viability of *S. dolichi* in culture slants was conducted. No growth was observed in the sub-cultures made from 195 days old cultures.

Thus it can be seen that the pathogen can survive at-least for 8 months in the infected leaves. Hence the pathogen can overwinter and infect the new crop in the next season.

Evaluation of fungicides

In-vitro evaluation:

Blitox, Difolation, Dithane M-45, Dithane Z-78, at 0.1, 0.2 and 0.3 per cent concentrations and Bavistin at 0.05, 0.1 and 0.2 per cent concentrations were tried adopting "poisoned food technique". The colony diameter was measured after 20 days of incubation and the results are presented in table XVIII.

From the results obtained it is clear that only Blitox at all concentrations was not effective in completely limiting the growth of the fungus. However no growth ws obtained in all the remaining fungicides at all concentrations tried. Thus it appears that 'poisoned food technique' might be unsuitable for slow growing fungi like Septoria.

Field evaluation of fungicides:

This experiment was conducted as described under "Material and Methods". The results obtained are presented in table XIX and fig. XII.

From the data, it is seen that number of diseased leaves per plant as well as severity of disease varied with the fungicide. Maximum control was obtained in the plots which received Difolaton sprays followed by Bavistin, Table. XVIII

Effect of fungicides on growth of <u>Septoria dolichi</u>.

Sl. No.	Fungicides	Concentration (%)	Mean colony diameter (mm)
1.	Dithane M-45	0.1	-
		0.2	-
		0.3	-
2.	Dithane Z-78	0.1	-
		0.2	-
		0.3	-
3.	Difolatan	0.1	-
		0.2	-
		0.3	-
4.	Bavistin	0.05	-
		0.1	-
		0.2	-
5.	Blitox	0.1	12.00
		0.2	8.33
		0.3	6.66
6.	Control	-	16.00

Table. XIX

Effect of fungicidal sprays on the disease development.

Sl. No.	Treatments	Average no. of leaves infected per plant (%)	Average leaf area affected per plant (%)
1.	Difolatan	23.32	0.64
2.	Bavistin	37.60	1.56
3.	Dithane M-45	39.15	2.33
4.	Dithane Z-78	48.72	2.91
5.	Blitox	61.47	9.41
6.	Control	65.12	11.12

Average no. of leaves infected/plant (%):

S.Em. ± 58.54

CD at 5% 11.52

Difolatan Bavistin DM-45 DZ-78 Blitox Control

Fig. XII. Effect of fungicidal sprays on the disease development.

1. Difolatan

2. Bavistin

3. Dithane M-45

4. Dithane Z-78

5. Blitox

6. Control

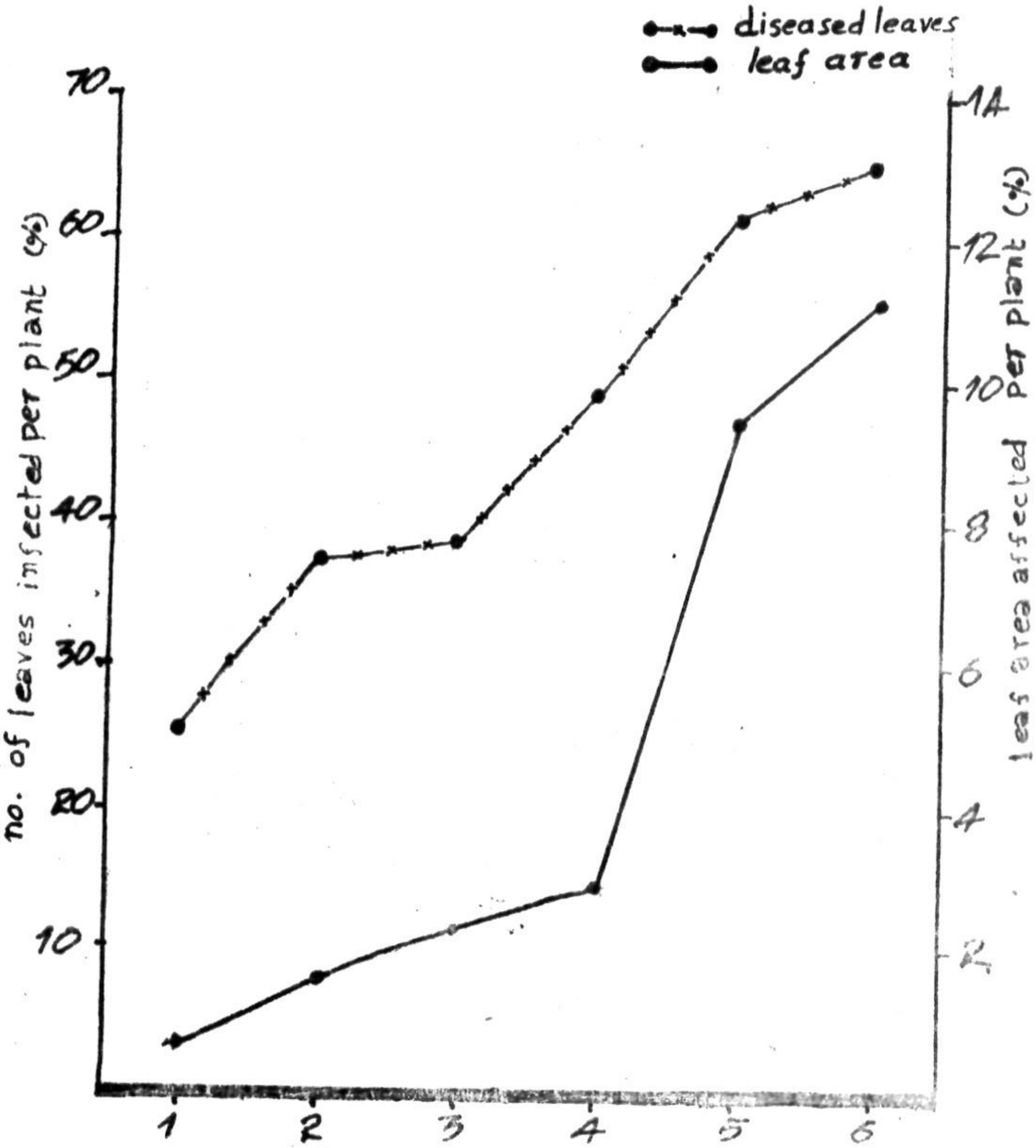


FIG XII EFFECT OF FUNGICIDAL SPRAYS ON THE DISEASE DEVELOPMENT

Dithane M-45 and Dithane Z-78. Blitox eas least effective. There was significant difference between the control obtained by Difolaton and the remaining fungicides. Further, Bavistin and Dithane M-45 and Dithane M-45 and Dithane Z-78 did not differed significantly. Blitox and the control plots also did not differ significantly with respect to number of diseased leaves per plant.

DISCUSSION

CHAPTER V

DISCUSSION

The results of the investigation on leaf spot disease of Dolichos lablab caused by Septoria dolichi are discussed in this chapter.

Morphological studies made indicated that there was very slight difference in conidial measurements of the isolate studied here as compared to the fungus described earlier by Berkeley and Curtis (Saccardo, 1884) and Chona and Munjal (1956). Since the difference in the conidial measurements could be influenced by many extraneous factors like the host, environment, this difference alone was not considered sufficient criteria for creating a new species. Hence the present isolate has been identified as Septoria dolichi Berk. and Curt. as reported by earlier workers.

Symptoms appeared on leaves, petioles and stem but not on pods. Further no sporulation was observed in the lesions on petiole, stem and midribs. Incubation period ranged from 11 to 18 days. Such a long incubation period appears to be common for most of the Septoria spp. (Weber, 1922a, 1922b and 1923).

Studies on growth phase of the fungus in potato dextrose broth indicated that the growth increased upto 33 days and thereafter started declining. This type of prolonged growth phase seems to be common for Septoria spp.

The fungus showed distinct difference in growth and cultural characters on different solid media. Maximum radial growth was obtained on Sabouraud's dextrose agar followed by seed extract agar and PDA. Least growth was recorded on Richard's agar and pod extract agar. Sabouraud dextrose agar, seed extract agar and PDA were preferred, probably due to significant amounts of several growth factors present in them. Maximum sporulation was also noticed on PDA and Sabouraud dextrose agar while sporulation was poor on pod extract agar. In most of the cases reaction of the fungus with the medium was dark except, in case of seed extract agar. Only on Sabouraud dextrose agar very much raised centre was observed. Growth of the fungus in different liquid media also varied. Maximum growth was recorded in seed extract followed by Sabouraud dextrose broth and PDB. Minimum growth was noticed in Asthana and Hawker's medium, while no growth was recorded in pod extract. This is in line with the findings of the earlier workers that Septoria spp. grow better on non-synthetic media (Weber, 1922a, 1922b; Arsenjevic 1965; Sohi and Sokhi, 1973; Endrinal and Celino, 1940; Kochman and Kubicka, 1962).

Studies on pod extract revealed that an alcohol soluble substance present on/in the pods inhibited the growth of the fungus. Since some growth was observed on pod extract agar, this substance appears to vary in concentration with the age of pods and weather conditions. This substance is oily in nature and is secreted by the pods which gives a peculiar aroma to the whole field. Sporulation was observed in the extracts of pods previously treated with alcohol. Further no sporulation was observed in the lesions on stem and petiole. Wilson (1961) found reduction in sporulation of Septoria on celery by oil sprays. Since the substance produced by the pods appears to be an oily substance, this substance appears to be produced in such concentrations which could inhibit the sporulation but not establishment, on petioles and stem. Further, spore germination studies on pods indicated that the germination is not inhibited. However the establishment of the fungus may be prevented. There are conflicting reports regarding the effect of several oils on spore germination of Mycosphaerella musicola. However, several workers are of the opinion that the oils act as therapeutants (Calpouzos, 1966). This might possibly explain the reasons for failure of pod infection. Specific studies with regard to the role of oil secreted by this plant, in infection and sporulation are necessary.

The fungus varied in its ability to utilize different carbon sources. Maximum growth was recorded on glucose followed by lactose, starch and sucrose. Relatively less growth was recorded on glycerol and rhamnose while no growth was recorded on sodium acetate and sodium citrate. Glucose, sucrose and maltose have been reported as good carbon sources for S. lycopersici (Sohi and Sokhi, 1973). Munjal and Gautam (1977) observed maximum growth of S. humuli on mannose, sucrose and glucose followed by maltose. Several strains of *Piricularia oryzae* are also known to make no growth on sodium citrate and sodium acetate as carbon sources (Otsuka, 1957). *Chytridium* sp. and *Pythiogeton* sp. make no growth on citrate and acetate form of carbon source. The inability of the fungus to utilize citrate and acetate form may be due to chelation of inorganic ions which may result in limitation of growth (Cochrane, 1958).

There was considerable variation in the utilization of different nitrogenous compounds used. Maximum growth was noticed on potassium nitrate followed by asparagine. No growth was recorded on aspartic acid and glutamic acid. These results agree with the observations on nitrogen utilization of *S. lycopersici* by Sohi and Sokhi (1973). Aspargine and glycine have been found to support good growth of *S. tritici* (Arsenjevic, 1966).

There was significant effect of different pH levels on growth of the fungus. Maximum growth was recorded at pH 6.1 followed by 6.6. No growth was recorded below 3.6. Similar optimal pH levels have been reported for *S.lycopersici* (Sohi and Sokhi, 1973; Patil, 1977) and *S. avenae* (Weber, 1922a). However, a still wider range of pH, 5.6 to 8.4 for *S. lycopersici* (Endrinal and Celino, 1940) and 2.5 to 9.2 for *S. tritici* (Weber, 1922b) also have been reported.

Temperature had a remarkable effect on growth and sporulation of the fungus. Maximum growth was recorded at 25°C followed by 20°C and 15°C. Very poor growth was observed at 30°C and 10°C while no growth was noticed at 5°C and 35°C. Similar results have been recorded for S. tritici (Weber, 1922b) and S. lycopersici (Patil, 1977).

Light also had determining effect on sporulation. Maximum sporulation was observed in cultures exposed either to continuous light or to an alternate cycle of light and darkness. Whereas in cultures kept in complete darkness sporulation was poor. These results are on par with those of Richards (1950) and Kurozawa and Balmer (1975) with S. nodrum and S. lycopersici, respectively.

Extent of spore germination was greatly influence by the nature of media used. Maximum germination was recorded in host extract after 48 hrs, followed by glucose and sucrose solution. Least germination was noticed in distilled water indicating nutritional requirement for rapid and maximum germination. Similar results were obtained by Patil (1977) and Luthra et al.(1937) while working with S. lycopersici and S. tritici, respectively. However, Shipton et al. (1971) who have reviewed the Septoria diseases of wheat state that both S. nodorum and S. tritici germinate well in water.

Temperature had a considerable effect on spore germination. Maximum germination was noticed at 25°C followed by

20°C and 30°C. No germination was recorded at 5°C and below while at 35°C less germination was observed. Similar type of temperature effect on spore germination of S. avenae has been reported by Weber (1922a).

The effect of relative humidity on spore germination was also significant. At 100 per cent relative humidity maximum germination was obtained after 48 hours while considerable amount of germination occurred at 98 per cent and 96 per cent relative humidity only after 120 hrs. Further no germination was observed below 96 per cent relative humidity even after 120 hrs. Spores of S. apiicola also reuire a minimum relative humidity of 96.8 per cent and above for germination (Sheridan, 1968). However Schnieder (1959) observed the minimum relative humidity for germination of S. obesa and S. chrysanthamella to be 92 per cent and 91 per cent, respectively. Thus under natural conditions, time required for infection may be delayed due to the requirement of high humidity for germination.

Studies on disease development indicated that both the incidence and severity of the disease are influenced by the weather conditions, mainly temperature, rainfall and humidity. During first 45 days, the mean temperature was above 24°C with 14 rainy days and the relative humidity was above 70 per cent most of the time. Even then the disease appeared only after 45 days. Further from 45 to 100 days the mean temperature ranged from 21.52 to 23.54°C with 25 rainy days and the relative humidity was above 74 per cent. During this period rapid increase in both the incidence and severity was noticed. After 100 days, the mean temperature ranged from 18.88°C to 21.27°C with no rainy days and the relative humidity was below 70 per cent. During this period decrease in disease incidence and severity was noticed in fourth and fifth sown crops, which was mainly due to the subsequent new foliage, not taking infection. Thus it appears that a mean temperature below 23°C with frequent rains and relative humidity above 75 per cent favours the disease development. The August sown crop was infected very late in the season and disease die not developed to severe form. While the September sown crop was infected severely. Since the favourable conditions are obtainable during October -November in Bangalore, it appears that September sown crop is more vulnerable to disease. However, conclusions can be drawn only after observing it for some more seasons. Similar requirements for disease development of several Septoria spp. have been reported. According to Buddin and Wakefield (1924 Septoria spp. attacking antirrhinum is favoured by cool and moist weather. Septoria lycopersici spreads widely during wet weather but checked by a period of prolonged dry weather (Reed, 1911).

Studies on mode of penetration indicated that S. dolichi enters the host through stomata. Several germ tubes were observed growing over stomata without penetrating them, which might be due to non-synchronization of growth of germ tubes and opening of stomata. Similar observations of stomatal penetration by Septoria lycopersici (Endrinal and Celino, 1940; Sohi and Sokhi, 1973) and Septoria glycines (Wolf, 1926) have been recorded.

Histochemical studies revealed that in the early stage of lesion formation, both protein and RNA appear to increase. However, this might be due to shrinkage of the width of leaf itself. Clumping of polysaccharide grains was also seen. In the final stage of lesion development, both RNA and protein became unstainable. This might be due to death of cells and break down of these compounds by the pathogen. Clumps of polysaccharide grains, in this stage became reduced in size and became sparse in the tissue. This might be due to utilization of these polysaccharides by the pathogen. Iodine-potassium iodide test showed that the polysaccharide present was starch. Further, starch has been found to be a good carbon source for S. dolichi. Thus it appears that the pathogen utilizes starch present in the host efficiently.

Several biochemical changes were observed in the leaf due to infection by S. dolichi. Total phenols and ortho-dihydroxy phenols were more in diseased leaves as compared to healthy leaves. Increase in phenols in diseased plants has been observed by Kiraly (1962), Raghunathan et al. (1966) and

Kishore and Chand (1975) and it appears to be a common phenomenon. Content of amino nitrogen also increased in diseased by McCombs and Winstead (1964), Raghunathan *et al.* (1966). This might be due to either break down of existing proteins or due to synthesis of amino acids without incorporating to proteins.

Host range studies have indicated that the pathogen *S. dolichi* is host specific and it will not infect most of the commonly grown pulses in Karnataka. Studies on perpetuation of the fungus in the diseased leaves and in culture have shown that *S. dolichi* can remain viable for more than six months. Since no pod infection takes place seed does not appear to carry over the infection. Since 'avare' is grown throughout the year and also grown as perennial crop, it is possible that both the infected debris and disease on the standing crop may serve as source of inoculum and may help in perpetuation of the fungus from one season to the next.

In-vitro evaluation of fungicides, indicated that Blitox even at 0.3 per cent concentration was not completely effective against *S. dolichi*. However the remaining four fungicides inhibited its growth at all concentrations.

The fungicidal trials conducted in the field, showed that Difolaton (0.2%) controlled the disease more effectively followed by Bavistin (0.05%), Dithane M-45 (0.2%) and Dithane Z-78 (0.2%). Blitox was found to be least effective. The results obtained suggest that three sprays with Difolaton (0.2%) could be successfully used in situations where the disease is a limiting factor.

SUMMARY

CHAPTER VI

SUMMARY

Investigations on leaf spot disease of *Dolichos lablab* caused by *Septoria dolichi* were carried out during 1977-78. The findings of these investigations are summarised below:

Morphological studies indicated that except for minor differences in conidial measurements the isolate under study, resembled the fungus described by earlier workers and hence the pathogen is regarded as *Septoria dolichi* Berk. and Curt.

The pathogen infects leaf, petiole and stem, but not pods. Sporulation was observed only on leaves. Different media used are found to exert varying effects on growth, sporulation and other characteristics. Maximum growth and sporulation were observed on Sabouraud dextrose agar followed by seed extract agar, while least growth and sporulation was observed on pod extract agar. Studies on growth phase showed that the fungus grows upto 33 days actively and then autolysis starts (in 10 ml PDB). Among the liquid media, seed extract supported maximum growth followed by Sabouraud dextrose broth. No growth occurred in pod extract. Further studies indicated that an alcohol diffusing substance present in the pods appears to inhibit the fungal growth.

Glucose was found to be the best carbon source for *S. dolichi* among the several sources of carbon tried. Sodium acetate and sodium citrate supported no growth. Among the various nitrogen sources tested potassium nitrate supported maximum growth followed by asparagine. No growth was observed in asparatic acid and glutamic acid.

The fungus grew over a range of pH from 4.1 to 7.8. Maximum dry mycelial weight was recorded at pH 6.1 followed by 6.6. It appears that fungus prefers acidic pH for its growth. However no growth was observed at and below pH 3.6.

The optimum temperature for growth and sporulation of S. dolichi was 25°C. At 10°C and 30°C very less growth was observed. The minimum temperature for growth ranged between 5°C to 10°C while maximum ranged between 30 to 35°C.

Light had a pronounced effect on sporulation of S. dolichi. Excellent sporulation was recorded in the plates exposed to continuous light or alternate light and darkness while no sporulation was observed in plates exposed to complete darkness.

Host leaf extract was found to be suitable medium for germination of spores while in distilled water minimum germination was recorded. The optimum temperature for germination in host extract was 25°C with the minimum ranging between 5 to 10°C and maximum being above 35°C. Maximum spore germination was observed at 100 per cent relative humidity and a minimum of 96 per cent relative humidity is required for germination within 120 hrs. Germination studies on pod indicated that germination is not inhibited but delayed.

Weather conditions had marked influence on the disease development. A mean temperature below 23.5°C with frequent rains and high relative humidity of above 75 per cent appears to be congenial for rapid development of disease in the field. Laboratory studies indicated that, minimum of 12 hrs duration of very high humidity is necessary for successful infection.

Host penetration of the fungus was found to be through stomata. Histopathological and histochemical studies indicated that in the early stages of lesion development apparent increase of proteins and RNA and clumping of polysaccharides were observed. However in the final stage of the leaf spot development, both proteins, RNA were undefectable while degradation of polysaccharides (starch) was noticed, indicating the utilization of these polysaccharides.

Several biochemical changes were observed in the leaves due to infection. Increase in total phenols, ortho-di-hydroxy phenols and amino nitrogen was observed in the infected leaves as compared to healthy leaves of the same age.

From the host range studies made, it appears that S. dolichi is host specific i.e., it is confined to D. lablab only. S. dolichi was viable upto 240 days in the form of spores in the diseased leaves and upto 195 days in cultures.

In-vitro evaluation of fungicides showed that Difolatan, Dithane M-45, Dithane Z-78 (all at 0.1, 0.2, 0.3%) and Bavistin (0.05, 0.1, 0.2%) completely inhibited the growth of S. dolichi at all the three concentrations. Blitox, even at 0.3 per cent concentration was unable to inhibit the fungus completely.

Fungicidal trials conducted in the field, showed that maximum control was given by Difolatan followed by Bavistin and Dithane M-45, while Blitox gave poor control. Efficacy of the fungicides under field trial was assessed on the basis of the total leaf area destroyed by the disease and number of leaves infected per plant in each treatment.

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CHAPTER VII

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