

**STUDIES ON MYCOPHYSIOLOGY OF BOTRYODIPLODIA
THEOBROMAE PAT. CAUSING MANGO (MANGIFERA
INDICA L.) TWIG BLIGHT AND ITS CONTROL**

**A THESIS
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE**

OF

Master of Science
(AGRICULTURE)

IN

PLANT PATHOLOGY

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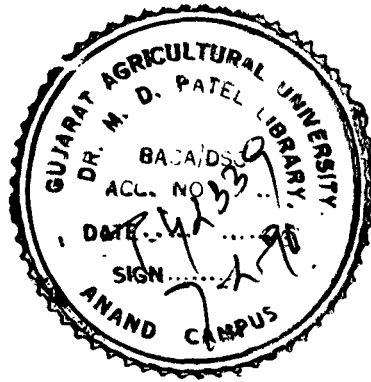
BY

GUNVANT A. PATEL

B.Sc. (Agri.)

**DEPARTMENT OF PLANT PATHOLOGY & BACTERIOLOGY
B. A. COLLEGE OF AGRICULTURE
GUJARAT AGRICULTURAL UNIVERSITY
ANAND CAMPUS, ANAND.**

SEPTEMBER, 1989



ABSTRACT

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STUDIES ON MYCOPHYSIOLOGY OF BOTRYODIPLODIA THEOBROMAE PAT.
CAUSING MANGO (MANGIFERA INDICA L.)
TWIG BLIGHT AND ITS CONTROL

Name of Student

Major Advisor

Shri G. A. PATEL

Dr. J.G. PATEL

Department of Plant Pathology,
B.A. College of Agriculture,
Gujarat Agricultural University,
Anand Campus, Anand 388 110

Twig blight with exudation of gum as suspected, proved to be the phasewise effect of mango decline, prevalent in South, Middle and North Gujarat. Botryodiplodia theobromae Pat. was always found associated and proved pathogenic. The pathogen also caused stem and lateral rot of the fruits.

The pathogen initially produced hyaline, one celled, thin walled, globose to subglobose immature spores, which after maturity turned brown black, two celled, thick walled and produced longitudinal striations. Pycnidial bodies were subglobose to globose, errumpent, ostiolated measuring $145 - 396.4 \times 107 - 323.5 \mu\text{m}$ with an average of $289.74 \times 219.13 \mu\text{m}$, while the average size of pycnidiospores measuring $26.4 \times 11.3 \mu\text{m}$.

Physiological studies indicated, Richard's medium to be the best for growth and sporulation. Maximum mycelial growth occurred at 15 day in incubation. Slightly acidic medium 6.0 to 6.5 pH and a temperature range between

25° - 30° C were optimum for both growth and sporulation. 'Glucose', 'Maltose', and 'Sucrose' were better and 'Lactose', 'Xylose', 'Galactose' and 'Starch' were inferior carbon sources while 'Calcium nitrate' as better and 'Ammonium sulphate' as poor nitrogen sources for the growth and sporulation. A combination of 'Calcium nitrate' as nitrogen and 'Maltose' as carbon proved significantly best. These sources could be replaced in Richard's medium against the respective normal sources.

Out of 12 fungicides tested in vitro, carbendazim (250 ppm) was most effective; while mancozeb, MEMC 6 %, PMA 1 % (1000 ppm, each) were next in order of merit, captafol, thiophanate methyl and aureofungin (2000 ppm, each) were little effective while carboxin and bordeaux mixture were ineffective.

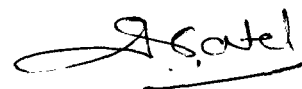
In the in vivo studies the fungicides and insecticide cum nematocide used as soil drenching carbendazim was found superior as compared to others.

Dr. J. G. Patel,
M.Sc. (Agri.); Ph.D.
Professor and Head,
Department of Plant Pathology
B. A. College of Agriculture,
Gujarat Agricultural University,
Anand Campus, ANAND - 388 110.

C E R T I F I C A T E

This is to certify that the thesis entitled,
"STUDIES ON MYCOPHYSIOLOGY OF Botryodiplodia theobromae
Pat. CAUSING MANGO (Mangifera indica L.) TWIG BLIGHT
AND ITS CONTROL" submitted by Shri Gunvantbhai A. Patel
in partial fulfilment of the requirements for the award
of the degree of Master of Science (Agriculture) in
Plant Pathology of the Gujarat Agricultural University
is a record of bonafide research work carried out by him
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(J. G. PATEL)
Major Advisor

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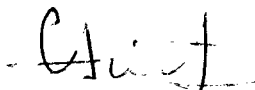
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CHAPTER-I
INTRODUCTION

The mango (Mangifera indica L.), family Anacardiaceae, is the most popular commercial orient fruit in India and occupies relatively the same position as the apple in temperate regions. Mango is believed to have originated in India and established since long. It is called king of the fruits. The tree is hardy in nature and has comparatively low maintenance costs.

The orchards well managed in all respects always give remunerative returns. Besides having a delicious taste, excellent flavour and attractive fragrance, it is an excellent source of vitamins A and C, sugar, acids, fat and protein, Kernel of mango contains carbohydrates, carotene, riboflavin, thiamine, protein, fat and calcium.

Of the total area under mango cultivation in the world, 70 per cent is occupied by India and hence considered to be the predominant country for mango cultivation. It is grown from southern east of India up to the border of the western desert as well as at sea level to 5,000 feet above in Himalayas (Bhatnagar and Subramanyam, 1973).

In India mango occupies 42.6 per cent of the total area under fruits comprising of 0.94 million hectares, with a total production of 8.21 million tons. Of the various states, U.P. has the largest area accounting for

nearly 33.2 per cent of the total followed by Bihar(13.5%) and Andhra Pradesh (13.4%) (Anonymous, 1985).

In Gujarat, it occupies more than 28,000 hectares area with a production of about 2,80,000 metric tons (Anonymous, 1982-83).

Mango is subject to attack by a number of diseases at all stages of its development right from the nursery to the fruit in storage (Singh, 1968). Almost every part of trunk, branch, twig, leaf, petiole, flower and fruit is attacked by fungi, bacteria and algae. Major diseases of mango are Bacterial leaf spot (Pseudomonas mangiferae, B.); Powdery mildew (Oidium mangiferae, Berth.); Anthracnose (Colletotrichum gloeosporioides Penz.); Malformation (Fusarium moniliformae, Sheld); stem-end rot of fruit (Aspergillus terreus Fr. Keissler); Twigblight (Macrophomina sp.) and Loranthus, a phanerogamic flowering parasitic plant. Some of the diseases take a heavy toll and have become limiting factors in mango orchards in certain regions. Out of all the diseases, mango die back and decline are observed to be quite destructive in India and many mango growing countries (Pathak, 1980). Since last decade, anthracnose and leaf blight are recorded to be the devastating diseases of mango, particularly alphanso orchards (Joshi, 1978). Patel (1978) reported heavy damage in mango orchards due to anthracnose (Colletotrichum gloeosporioides, Penz.) while leaf tip

blight (Phyllosticta sp.) was found to be the cause of heavy leaf fall.

However, the incidence of leaf infecting fungi continues to increase year after year resulting into decline accompanied by exudation of gum and die-back of mango orchards. Therefore, it can be considered as a destructive disease and limiting factor for mango cultivation in South, middle as well as North Gujarat. The increase in inoculum potential may lead to destruction of the orchards. Twig blight appear to be the initial phase of die-back and decline accompanied by exudation of gum which are the major symptoms with which Botryodiplodia sp. was always found to be associated. The problem was, therefore, taken-up on hand to ascertain the real cause of disease and to study the morphology of causal agent, symptomatology, physiological requirement such as superior media for excellent growth, optimum pH, temperature, sources of carbon and nitrogen and use of effective chemicals in managing this devastating disease.

**REVIEW
OF
LITERATURE**

CHAPTER - II
REVIEW OF LITERATURE

2.1 INTRODUCTORY :

Mango is attacked by 75 fungal, 5 bacterial, 1 algal and other phanerogamic parasites (Singh, 1968; Joshi and Desai, 1979). Botryodiplodia theobromae Pat. was initially reported to cause rot of cacao fruits, from Ecuador by Patounillard and Lagerhim (1892); however, it has also been found to be responsible for die-back with exudation of gum on twigs of mango. Pertinent literature regarding Botryodiplodia theobromae Pat. on mango have been presented here.

2.2 OCCURRENCE :

Botryodiplodia sp. was initially reported to be causing die-back of mango from India (Das Gupta and Zachariah, 1945); Puerto Rico (Alvarez Garcia, 1967) and Egypt (Ragab et al., 1971).

In India it was reported from Uttar Pradesh (Das Gupta and Zachariah, 1945); Jobner (Rajasthan), (Verma and Singh, 1970, 1973, 1974); Bhubaneshwar (Orissa) (Swain, 1974; Mohanan, 1975; Rath et al., 1978) and Punjab (Chadha, 1978).

Fruit rot caused by B. theobromae was reported from Delhi (Chakravarti and Srivastava, 1964; Dharam Vir et al., 1967; Vyas, 1986), Allahabad (Ehargava et al., 1966;

Srivastava and Tandon, 1969), Orissa(Sohi et al., 1973; Rath and Mohanan, 1977, 1986) and Surat (Gujarat) (Uppal et al., 1934).

Mostly all the varieties including 'Mohanbhog', Deshi, Dashehari, Langra, Saroli and Safeda are more or less susceptible to stem end rot of fruit caused by B. theobromae but, 'Mohanbhog' is the most susceptible and 'Saroli' the least (Verma and Singh, 1973).

2.3 SYMPTOMATOLOGY :

Botryodiplodia theobromae Pat. attacks blossom, branches, panicles, twig, pedicel and fruits of the plant and produces various types of symptoms which are described as under :

Leaf blight :

The upper leaves lose their healthy green colour and gradually turn brown to dark brown. Darkening advances towards the tip above the infected region. The browning also starts at the base of the leaf which spreads along the midrib and veins to the margin. This is followed by browning of the whole leaf accompanied by the upward rolling of the margin (Rath et al., 1978).

Wither tip :

Symptoms are restricted to the tender tips of the one year growth. The tips dry up, lose weight, develop cracks and turn brown. The associated leaves turn pale,

dry up and ultimately drop-off leaving distinct leaf surface (Rath et al., 1978).

Blossom blight :

The presence of small, dark coloured lesions on the panicles, branches and pedicels is very common. As a result flowers turn brown, wither and drop-off (Rath and Mohanan, 1977).

Twig blight :

The affected leaves show browning of the entire lamina, eventually the affected twig or branches dry up and shrivel. The brown rolled leaves often persist for a month or more and finally drop off leaving the shrivelled twig (Rath et al., 1978).

Die back :

Rath et al. (1978) found that in die back phase, large branches and basal trunk develop bark cankers and gummy exudations. Eventually even large branches of the entire tree die. Pathak (1980) showed that, the onset of die back become evident by discolouration and darkening of the bark at some distance from the tip. The dark area advances and young green twigs slowly and slowly start withering first at the base and then extending upwards from the base, subsequently extending along the veins of leaves. The affected leaves turn brown and roll upward. At this stage, the twigs or branches dry and shrivel.

This may be accompanied with exudation of gum. Burhan(1987) observed the blackening and necrosis of the tap root in *Botryodiplodia* die-back infected seedlings of mango. He also noticed die-back, twig blight and blackening of stem, canker, necrosis and root necrosis.

Sharma and Sankaran (1988) showed that die-back caused by *Botryodiplodia theobromae* is characterised by development of stem canker, gradual die-back of shoots and finally death of trees due to girdling of stem by progressive cankers.

Fruit rot :

Stem-end rot or rough rot or storage spoilage starts from stem-end portion, showing dark brown to black discoloration around the stem end. Under humid condition, the fruit turns completely black and pulp becomes light brown and soft (Quimino and Quimino, 1974). Vyas (1986) observed that in the primary stage, small light brown patches on the fruit on the development of the disease the colour of the spots changed to dark-brown and finally to black. With the advancement of rot, small dark brown to blackish pycnidial bodies appeared on the infected regions and later the fruit became pulpy.

2.4 Isolation :

Uppal et al. (1934) isolated *B. theobromae* Pat from mango fruit. While Das Gupta and Zachariah (1945)

isolated it from mango twigs which was associated with Phoma sp. and Fusarium sp. Rath and Mohanan (1977) isolated B. theobromae from mango twig and fruit causing blossom blight which was associated with Colletotrichum gloeosporioides Penz. Fusarium Sp., Aspergillus sp., Pestalotia mangiferae (P. Henn.); (Curvularia maculans Bancroft) Boedijn; Sclerotium rolfsii Sacc. and sterile mycelium. Further, Rath et al. (1978) also reported association of Pestalotia mangiferae (P. Henn); Phoma sp., Colletotrichum gloeosporioides Penz., Sclerotium rolfsii Sacc., Rhizoctonia solani, Kuhn; Aspergillus niger Van Tiegham; Cladosporium cladosporioides (Ires) De vries; Curvularia maculans (Bancroft) Boedijn; Alternaria tenuis Auct.; Peziotrichum corticola (Massec) Subram, and two unidentified species. Rowan (1982) reported association with Fusarium moniliformae (Gibberella fujikuroi J.). Mattos and Ames (1986) isolated from trees with defoliation, die-back, basal rot and cankers on the trunk and branches of the apple and identified as Botryodiplodia theobromae. Adisa and Obinyereokwa (1988) reported association of B. theobromae with Rhizopus stolonifer and Trichoderma sp.

2.5 PATHOGENICITY TEST :

Principally die-back and twig blight of mango caused by B. theobromae and association of several microorganisms enhance the infection (Rath et al., 1978).

Rath and Mohanan (1986) found Botryodiplodia theobromae Pat., pathogenic to mango in artificial inoculation test and reported that it incites fruit rot of mango.

Lutchmeah (1988) isolated B. theobromae from severely rotted fruits in Mauritius and it was shown to be pathogenic to wounded fruits with severe rotting under controlled conditions.

2.6 MORPHOLOGY OF THE PATHOGEN :

Olunloyo and Esuraoso (1975) isolated the pathogen Lasiodiplodia theobromae (Botryodiplodia theobromae from apple and indicated that it is imperfect stage of Botryosphaeria rhodina. Alvarez Garcia and Lopez (1971) reported serious die-back in grafted mango due to Physalospora rhodina, the perfect stage of Diplodia. This indicated that Botryodiplodia theobromae is the imperfect stage of Botryosphaera rhodina.

Chona (1933) stated that Botryodiplodia is a very fast growing fungus and has profuse aerial mycelium which is cottony white to start with, but gradually turns greenish-grey and finally dark-grey when the cultures are about three weeks old. Countless tiny black pycnidia partly embedded in the medium are formed. Black sclerotial bodies are formed frequently in old cultures.

The pycnidia of Botryodiplodia sp. were aggregated in botyose clusters, thin walled, single celled and

hyaline immature conidia, later it produced 2-celled dark brown, anallides dolliform to shortly cylindrical mature conidia (Ainsworth et al., 1973). Pycnidia originated from a net work of vegetative hyphae (Ekundayo and Haskins, 1969). Pathak (1980) found that Diplodia natalensis, Evans produced brown to black, globose to subglobose, pyriform or erumpent pycnidia which were ostiolate. Prabhakara Rao and Deshmukh (1986) stated that pycnidia were gregarious, black erumpent, globoid to irregular, occasionally ostiolate, measuring 135.0 - 398.5 μ X 100.0 - 315.0 μ with an average of 286.8 X 217.5 μ . The young pycnosporos are hyaline and one celled becoming dark coloured and bicelled at maturity (Barnett and Hunter, 1972). The spores are oblong to ellipsoidal and measuring on an average 28.0 X 10.9 μ (Prabhakara Rao and Deshmukh, 1986); 24.0 to 30.0 μ X 11.5 to 23.5 μ (Ekundayo and Haskins, 1969); and 24.0 X 12 to 16 μ (Luke and Paul, 1982).

2.7 PRIMARY INOCULUM :

Verma and Singh (1970) found that B. theobromae could survive in infected and dead mango twigs even in hot weather. Pathogen also survives under favourable conditions by means of chlamydospores and specialized hyphae in diseased Yam tissue (Ogundana, 1983). Aderiyee and Ogundana (1986) stated that pathogen can also survive in the form of chlamydospores and microconidia.

2.8 PENETRATION :

Chakravarti and Srivastava (1964) found Diplodia natalensis Evans to penetrate the mango fruit near the stem-end only when it was wounded. Pathak and Srivastava (1967) and Lele et al. (1975) showed that the pathogen travels and invades through vascular system of the pedicel and thus infects the uninjured fruits. Verma and Singh (1974) reported that the fungus could enter the host through wounds inflicted either by insects or by primary injury. Infection of the B. theobromae starts through the wound (Ogundana, 1983; Sharma et al., 1984 and Sharma and Sankaran, 1988).

2.9 EPIDEMIOLOGY :

Verma and Singh (1973) reported that the mango variety 'Mohanbhog' is the most susceptible to this disease, while variety 'Saroli' is least. None of the varieties were resistant to this disease. Rath and Mohanan (1977) observed severe infection of Botryodiplodia theobromae on panicle axis of mango tree, while Verma and Singh (1974) reported that young branch and ripened fruits were more vulnerable. Rath et al. (1978) observed maximum infection on 3 to 12 years old mango plant. Nutritional deficiency predisposed the plant to this disease. The disease was severe when B. theobromae was associated with other organisms. Vyas (1986) reported that the maximum incidence of die-back in August or during the

period of higher humidity. The maximum growth of B. theobromae and disease development under field conditions were recorded at 25.9 to 31.5°C. (Verma and Singh, 1970). Rath and Mohanan (1977) also mentioned that the blossom blight caused by B. theobromae was maximum under the conditions of heavy rainfall.

Botryodiplodia theobromae has been reported to occur on 42 hosts as mentioned by Butler and Bisby (1960); Sarbhoy et al. (1975) and Bilgrami et al. (1979).

2.10 PHYSIOLOGY OF THE PATHOGEN :

2.10.1 Media :

Siradhana (1960²) reported that the Richard's medium was the best medium for the maximum growth of papaya isolate of B. theobromae. Verma (1968) observed that Glucose-asparagin medium was the best followed by Richard's medium for maximum growth. He also noticed that Asthana and Hawker's medium gave poor growth. Bhatnagar (1970) reported that the Czapek's medium showed the best growth, while in Asthana and Hawker's medium growth of fungus was very poor. Patel (1971) stated that Yam-leaf-decoction-agar proved to be the best followed by Richard's medium for the good growth of B. theobromae (Yam isolate).

2.10.2 Temperature :

Srivastava and Tandon (1963) showed that the fungus could grow between 15° to 35° C temperature.

They also noticed that it did not grow at 10° and 40° C. Srivastava (1968) observed that optimum temperature for growth and sporulation was 25° C but conidial germination was maximum at 30° C. Tsai (1983) reported that the mycelial growth of an isolate of banana (B. theobromae) in vitro was best at 25° to 30° C and pycnidial formation at 30° C. Seehann and Tabirih (1983) and Mattos and Ames (1986) observed that 25° to 30° C was optimum temperature, for the best growth and pycnidial production of B. theobromae.

2.10.3 Hydrogen-ion concentration :

Patel (1971) stated that the fungus could grow in a wide range of pH (3.5 to 8.5); the best growth was near neutral or acidic condition and it decreased when pH value either increased or decreased. Optimum growth of the fungus was obtained at pH 6.0 (Srivastava, 1968). Nwufo and Fajola (1986) stated that maximum growth of the fungus was between pH of 6.0 - 7.0. Tsai (1983) observed poor growth of the fungus (B. theobromae) at pH 2 - 3.

2.10.4 Carbon and Nitrogen sources :

Margolin (1940) and Seehann and Tabirih (1983) found Sucrose and Glucose to be the best carbon sources for the growth of the fungus. While, Lactose was a poor carbon source. Monosaccharides viz., D. fructose and Glucose were found to be good carbon sources (Bhatnagar, 1970; Verma and Prasad, 1975; Kaul, 1987); While Dextrin

and Starch (Srivastava and Tandon, 1969), Galactose and Sorbose (Verma and Prasad, 1975); Rhannose and Mannitol (Kaul, 1987) were poor carbon sources for the growth of B. theobromae.

Among nitrogen sources, mixture of amino acid (Prasad, 1968; Srivastava and Tandon, 1969), Ammonium phosphate, Ammonium nitrate (Patel, 1971); Calcium nitrate (Kaul, 1987) gave maximum growth; while Ammonium chloride and Sodium nitrate (Patel, 1971; Sreeemali, 1973; Tsai, 1983), Ammonium chloride and Glycerine (Lal, 1969), Sodium nitrate (Tsai, 1983; Kaul, 1987) gave poor growth of B. theobromae.

2.10.5 Light and moisture :

Schmidt and Fergus (1962) stated that light was necessary for pycnidial production. Perera and Lago (1986) found that under 8,000 lux, mycelial growth and pycnidia formation of Diplodia natalensis (B. theobromae) were best with daily photoperiods of upto 16 hrs, sporulation being inhibited with less than four hours light daily over 32 days. Ekundayo (1970) found that maximum spore germination at 100 per cent moisture. Verma and Singh (1970) stated that maximum development of disease was observed at the time of highest humidity (83.6 % per day). Pathak (1980) reported that the relative humidity above 80 per cent favours the disease.

2.11 MANAGEMENT :

2.11.1 In vitro :

Srivastava and Tandon (1971) observed that Captan @ 1.0 per cent was effective against guava and mango isolates of B. theobromae. They showed that complete inhibition of spore germination occurred at all the concentrations of the antibiotic 8-Hydroxyquinoline benzoate (Nystatin) at 200, 300 and 400 ppm. Raghvan and Saksena (1978) showed that Captan gave cent per cent growth inhibition at 0.2, 0.5, 1.0 and 2 per cent and PCNB (Brassicol) at 1 and 2 per cent. Kumbhare and Chaudhari (1979) evaluated five fungicides and the best control was obtained by benomyl (Benlate) and carbendazim (Bavistin) at the concentration of 0.01 per cent. Pandey et al. (1980) tested dithianon (Delan) and triforine (Saprol 15 EC) at 1000 and 1250 ppm which effectively prevented the growth of B. theobromae. Splading (1982) reported that the CGA 64251 (Sonax) was most effective for the control of post-harvest decay (B. theobromae) in mango cvs., Tommy Atkins and Keitt. Anusuya (1984) tested five fungicides against stem canker of mulberry caused by B. theobromae and found that carbendazim (Bavistin) and captafol (Difolatan) were most inhibitory to growth of the fungus in vitro being effective only at 100 ppm. Ekundayo (1984) reported that 6-methylpurine prevented production of pycnidia and inhibited the germination of mature pycnidiospores but had no apparent effect on

mycelial growth of Lasiodiplodia (Botryodiplodia theobromae). Fajola and Nwufo (1985) observed that spore germination and mycelial growth of the fungus were completely inhibited by captan and benomyl (Benlate) each at 100 to 500 ppm. Ritcher and Kone (1986) showed that hot-water treatment for 30 minutes was initially highly effective against conidial germination on citrus fruit-rot caused by B. theobromae in vitro. Vyas and Singh (1977) found that apple isolate of B. theobromae was controlled by dipping in carbendazim (Bavistin) at 500 ppm or TBZ (Thiabendazole) and aureofungin at 1000 ppm. Raghavan et al. (1978) found that aureofungin was most effective against B. theobromae, followed by nystatin and griseofulvin. Atri (1985) while evaluating three antibiotics against Botryodiplodia rot of pear showed that aureofungin, nystatin and talsutin (1000 ppm each) caused cent per cent inhibition of mycelial growth but only nystatin could effectively control the fruit rot in vitro. Decay of mango fruits caused by B. theobromae was prevented by storage at 10°C (Bhargava et al., 1966). Srivastava and Tandon (1968) suggested careful handling, storage at 15°C, quick transport and sale for controlling fruit rot caused by B. theobromae. Ogawa et al. (1968) observed that best control was obtained with a dip or by painting with benomyl (Benlate) at 1600 ppm + surfactant f. Basofix EM 117, mancozeb

(Dithane M-45) and captan. Tsai (1969) reported that decay by B. theobromae on banana was inhibited when fingers were dipped for three second in 1600 ppm, benomyl (Benlate) and held eight days at 15° C. Ogundana and Dennis (1981), evaluated 12 fungicides and found that only benomyl (Benlate) and thiobendazole (Martect) completely prevented the infection.

2.11.2 In vivo :

Pathak (1980) stated that in the orchard, good control was achieved by spraying with copper oxychloride and painting stems with the same fungicide.

Olunloyo (1983) reported that captafol(Difolatan) 1.5 g a.i./l + $\sqrt{\text{-BHC}}$ 1 g a.i./l was most effective against cashew disease caused by Lasiodiplodia (Botryodiplodia theobromae) in preventing inflorescence infection and increasing nut yield. Mattos and Ames (1986) showed that in the glass house, of the various fungicides applied as paste on apple cuttings and grafts infected with B. theobromae only benomyl (Benlate) gave some control.

Utikar et al. (1986) evaluated eight fungicides under field condition and observed that benomyl(Benlate), carbendazim (Bavistin), copper oxychloride (Blitox-50) and mancozeb (Dithane M-45) gave some control of fruit-rotting (B. theobromae Pat.) of pomegranate.

MATERIALS
&
METHODS

CHAPTER - III
MATERIALS AND METHODS

Materials and methods followed during the course of investigation are furnished below.

3.1 PATHOLOGICAL INVESTIGATIONS :

Collection of samples

Naturally infected mango twigs were collected from the orchard of horticultural farm, B.A. College of Agriculture, Anand and examined visually and microscopically. The symptoms and signs observed were recorded.

Isolation of the organisms

Selected diseased twig of mango was thoroughly washed to remove the dirt and soil. The blighted portion of the twig was cut into small pieces. The same pieces were kept for two minutes in 0.1 per cent mercuric chloride solution for disinfection and then washed thrice with sterilized distilled water. After washing, the pieces were placed on potato-dextrose-agar (PDA) in plates. The petriplates were incubated for one or two days at $28^{\circ} \pm 1^{\circ}$ C temperature. The mycelial growth from pieces were transferred aseptically on PDA slants. The fungus thus isolated was maintained for further use in pure culture on potato-dextrose-agar.

3.2 PATHOGENICITY TEST :

Inoculum from about 12 days old culture grown on liquid media was prepared in sterile water. Mycelial and conidial suspension thus prepared by thoroughly mixing in homogenizer was used for inoculation on seedlings and fruits of mango.

3.2.1 Seedling inoculation :

Healthy mango seedlings were raised in the glass-house. The stems of the one year old seedlings were surface sterilized with 0.35 per cent sodium hydrochloride^t solution and washed subsequently with sterilized distilled water. An area of two cm of the stem was marked and inoculated. Sterilized cotton swab was made and dipped into the inoculum and then inoculum was put on the injured (with the help of scalpel) and uninjured surface of the seedlings. The control seedlings were treated similarly with sterile water without fungal inoculum. All the inoculated and uninoculated seedlings of mango were kept in separate moist (95 % RH) chamber at room temperature ($28^{\circ} \pm 1^{\circ} \text{C}$). The initiation and development of symptoms were observed daily and recorded.

3.2.2 Fruit inoculation :

Healthy fruits (Var. Deshi) were collected, sterilized with 0.35 per cent sodium hypochloride^t solution and washed subsequently with sterile water. About two cm area at the middle and at stem-end portion of the fruits

were marked and conidial suspension (30 - 55 conidial per low power (10 X) microscopic field). Uninoculated fruits served as control. In all four replications each with two fruits placed under moist chamber (desiccators filled with water at the base) at room temperature. Initiation and development of symptoms were observed daily and recorded accordingly.

3.3 IDENTIFICATION OF THE PATHOGEN :

The pure culture of the fungus, obtained through single spore isolation after proving pathogenicity, was sent to International Mycological Institute, Kew, Surrey, England for identification which was identified as Botryodiplodia theobromae Pat. (IMI - 32 22 70). The culture isolated from artificially inoculated twigs and fruits was identical to the original isolate.

3.4 MORPHOLOGY AND PHOTOGRAPHS :

Fungal growth was examined under the microscope. Photographs of hyphae, pycnidiochore, pycnidia and conidia were taken with the help of a research microscope having photo-automatic arrangement. The size of 100 randomly selected pycnidia and conidia were measured with the help of stage and ocular micrometers.

3.5 MYCELIAL GROWTH :

Erlenmeyer flasks (100 ml) containing 20 ml of liquid medium were sterilized at 1.2 kg/cm^2 pressure for 20 minutes then inoculated with uniform bits of 0.5 cm inoculum cut with the help of sterilized cork borer

from 12 days old culture grown on potato-dextrose-agar. The flasks were then incubated for 15 days at room temperature ($28^{\circ} \pm 1^{\circ}\text{C}$). The mycelial mat in the liquid media was collected by filtration on pre-weighed Whatman's filter paper No. 42. Dry weight was taken on monopan balance after drying at constant temperature (60°C) for 24 hours in a hot-air oven.

3.6 PHYSIOLOGICAL STUDIES :

3.6.1 Search for best media :

With a view to select the best medium for Botryodiplodia theobromae Pat, following liquid media were evaluated in the present study.

1. Asthana and Hawker's medium.
2. Brown's medium.
3. Czapek's medium.
4. Elliott's medium.
5. Modified Asthana and Hawker's medium.
6. Potato dextrose broth.
7. Potato sucrose broth.
8. Richard's medium.
9. Standard nutrient medium.

Details of the ingredients used in preparation of these media are mentioned in Appendix-I. The pH of each medium was adjusted to 6.0 on a pH meter, using 0.1 N HCl or 0.1 N NaOH before autoclaving. Erlenmeyer flasks(100 ml)

containing 20 ml of respective liquid media were sterilized at 1.2 kg/cm^2 pressure for 20 minutes and then inoculated with uniform bits of 0.5 cm inoculum cut with the help of a sterilized cork borer from the margin of 12 days old culture on potato-dextrose-agar. The flasks were then incubated for 15 days at room temperature ($28^\circ \pm 1^\circ \text{C}$). Four replications were maintained for each medium. The mycelial mat was collected from the medium by filtering through previously weighed Whatman's filter paper No. 42, washed twice with distilled water, dried at 60°C for 24 hours and weighed.

3.6.2 Effect of incubation period on the growth of *Botryodiplodia theobromae* Pat. :

To determine the incubation period, seven incubation periods viz., 5, 10, 15, 20, 25, 30 and 35 days were selected and replicated four times. Harvesting of the mycelial growth in each was done at the end of incubation period. The incubation period at which maximum mycelial growth was obtained was selected further for physiological studies.

3.6.3 Effect of different temperature on the growth of *Botryodiplodia theobromae* Pat. :

Among various synthetic media, Richard's medium was found better and hence selected as basal medium. The growth of the fungus was studied over a wide range of

temperature to determine the optimum temperature requirement of the fungus. Various degrees of temperatures viz., 5, 10, 15, 20, 25, 30, 35 and 40°C were adjusted in separate incubators. Twenty ml of Richard's medium was taken in 100 ml flasks sterilized at 1.2 kg/cm² pressure for 20 minutes. They were placed in incubator for 15 days at respective temperature after inoculating with 0.5 cm diameter disc of 12 days old fungus culture. The mycelial mat was collected from the medium by filtering through pre-weighed Whatman's filter paper No. 42, washed twice with distilled water, dried at 60°C for 24 hours and weighed.

3.6.4 Effect of hydrogen-ion concentration on the growth of *Botryodiplodia theobromae* Pat.

For studying the effect of pH on the growth of *B. theobromae* Pat., it was grown in Richard's medium at pH 4.0, 4.5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Various pH levels were adjusted by adding 0.1 N HCl or 0.1 N NaOH before autoclaving the medium. Flasks containing 20 ml of basal medium at respective pH values were inoculated with 0.5 cm diameter disc of 12 days old culture. Four replications were maintained for each pH level and were incubated at room temperature (28° ± 1°C) for 15 days. Dry mycelial weight as well as change in pH value was also recorded as per the procedure described earlier.

3.6.5 Effect of various carbon sources on the growth of Botryodiplodia theobromae Pat. :

Equivalent weight of carbon in Richard's medium was replaced by different carbon compounds. The quantity of carbon sources added was determined on the basis of their molecular weight. The final concentration was equal to the amount of carbon present in 50 g of sucrose per litre of the medium, except that in case of polysaccharides where the amount of the chemical used was equal to the weight of sucrose incorporated.

The various sources of carbon compounds used in Richard's medium are shown below :

Group	Source	Chemical formulae	Mol. wt.	% of carbon
I Monosaccharides	1. Fructose	$C_6H_{12}O_6$	180	40
	2. Galactose	$C_6H_{12}O_6$	180	40
	3. Glucose	$C_6H_{12}O_6$	180	40
	4. Xylose	$O-CH_2(CH_2OH)_3CHOH$	150	40
II Disaccharides	5. Lactose	$C_{12}H_{22}O_{11}$	342	42
	6. Maltose	$C_{12}H_{22}O_{11}$	342	42
	7. Sucrose	$C_{12}H_{22}O_{11}$	342	42
III Polysaccharides	8. Starch	-	-	-
IV Control	9. No carbon	-	-	-

The measured quantity of different carbon compounds were dissolved in the medium. The pH of the medium was adjusted to 6.0. Twenty ml of liquid medium was filled in each of the 100 ml flasks replicated four times. These were sterilized at 1.2 kg/cm^2 pressure for 20 minutes. Flasks were then inoculated with 0.5 cm diameter disc of the inoculum obtained from 12 days old fungus culture. They were incubated at room temperature ($28^\circ \pm 1^\circ \text{C}$) for 15 days. Dry weight of mycelial mat, pycnidial production and changes in pH value were recorded.

3.6.6 Effect of various nitrogen sources on the growth of *B. theobromae* Pat. :

Preference to utilise particular source of nitrogen was determined by the amount of growth of the pathogen in Richard's medium, replacing potassium nitrate by nitrogen compounds. The amount of each chemical used was calculated on the basis of the molecular weight so that the total nitrogen remained the same per litre of medium. Thus, the amount of nitrogen used in different sources was equivalent to the amount of nitrogen available in ten g of potassium nitrate. Various nitrogen sources tried in Richard's solution were as under :

Source	Chemical formulae	Mol. Wt.	% of nitrogen
<u>Inorganic</u>			
1. Ammonium acetate	$\text{CH}_3\text{COONH}_4$	77.08	18.00
2. Ammonium nitrate	NH_4NO_3	80.04	35.00
3. Ammonium oxalate	$(\text{COONH})_2 \cdot \text{H}_2\text{O}$	142.11	19.70
4. Ammonium phosphate	$(\text{NH}_4)_2\text{HPO}_4$	131.00	16.00
5. Ammonium sulphate	$(\text{NH}_4)_2\text{SO}_4$	132.14	21.20
6. Calcium nitrate	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	263.13	75.50
7. Potassium nitrate	KNO_3	101.11	13.84
8. Sodium nitrate	NaNO_3	85.00	16.50
<u>Organic</u>			
9. Urea	$\text{NH}_2\text{CO NH}_2$	60.00	46.66
10. Control (without nitrogen)	-	-	-

The measured quantities of different nitrogenous compounds were dissolved in the medium and the pH adjusted to 6.0. After preparation 20 ml liquid medium was taken in each of the 100 ml flasks, replicated four times. These were sterilized at 1.2 kg/cm^2 pressure for 20 minutes. Flasks were then inoculated with 0.5 cm diameter mycelial disc obtained from 12 days old culture. They were incubated at room temperature ($28^\circ \pm 1^\circ \text{C}$) for 15 days. The dry weight of mycelial mat, and pycnidial production were recorded.

3.6.7 Effect of various combinations of 'Carbon' and 'Nitrogen' sources in Richard's medium on the growth of *Botryodiplodia theobromae* Pat.

Richard's medium was found to be best for growth of *Botryodiplodia theobromae* Pat. isolated from mango.

Sucrose, Glucose and Maltose which proved to be the good carbon sources and calcium nitrate, Urea and potassium nitrate as better nitrogen sources were included in this study. In all, nine combinations of carbon and nitrogen sources were tested with the rest of the ingredients as in Richard's medium. Twenty ml medium of each combination was filled in each of the 100 ml flasks, replicated four times.

Various combinations of 'Carbon' and 'Nitrogen' sources replaced in Richard's medium are as under.

Sr. No.	Carbon sources	Nitrogen sources
1.	Glucose	Calcium nitrate
2.	Glucose	Potassium nitrate
3.	Glucose	Urea
4.	Maltose	Calcium nitrate
5.	Maltose	Potassium nitrate
6.	Maltose	Urea
7.	Sucrose	Calcium nitrate
8.	Sucrose	Potassium nitrate
9.	Sucrose	Urea

The flasks were sterilized, inoculated and incubated for 15 days as per the procedure described earlier. Dry mycelial weight, final^{al} pH and pycnidial production were recorded in the same manner as were used earlier.

3.7 EVALUATION OF SYSTEMIC AND NON-SYSTEMIC FUNGICIDES AGAINST BOTRYODIPLODIA THEOBROMAE PAT. IN VITRO :

Twelve systemic and non-systemic fungicides (mentioned in Appendix-II) were tested in vitro against B. theobromae Pat. by 'paper disc method' using PDA as a basal medium.

Twenty ml of sterilized hot (45° to 50° C) PDA medium was poured in each sterilized petriplates and allowed to solidify. After solidification, the plates were inoculated with two to three drops of spore suspension of 12 days old culture of test fungus. Two paper disc (5 mm diameter) previously impressed with the test fungicides were placed on PDA plates seeded with spores. The fungicides used were tested at 250, 500, 1000, 2000 and 4000 ppm concentrations. The petriplates were incubated at room temperature (28° ± 1° C). Four replications were maintained for each fungicide. Mean inhibition zone was recorded when clear zone of inhibition around each disc appeared. The inhibition zones were calculated by subtracting the diameter of disc from total inhibition zone.

3.8 EFFECT OF VARIOUS CHEMICALS ON INTENSITY OF THE DISEASE
CAUSED BY B. THEOBROMAE IN FIELD/ORCHARD :

The fungicides which were found effective during in vitro studies ^{were} ~~are~~ further used in combination of nematicide cum insecticide against the control of Botryodiplodia die-back or gummosis at Mango Orchard, B.A. College of Agriculture, Anand.

Three fungicides and one nematicide cum insecticide viz., Bordeaux mixture (@ 1 %), carbendazim (Bavistin 50 WP @ 0.05 %), FMA (FMA 1 % @ 0.001 %), carbofuran (Furadan 3 G @ 50 g/tree) and combination of each fungicide with a nematicide cum insecticide (Furadan 3 G) were selected for soil drenching application to mango trees. For soil drenching of each fungicide 20 lit. of water/tree was used while for granular application of nematicide cum insecticide carbofuran (Furadan 3 G) @ 50 g/tree dose was used.

Trial details :

- (1) Location : Mango orchard at B.A. College of Agriculture, Anand.
- (2) Date of treatment application :
 - (i) January 1, 1989.
 - (ii) April 6, 1989.
 - (iii) July 4, 1989.

(3) Treatments :

- | | | |
|-----------------------------------|---|----------------|
| (a) Bordeaux mixture | : | 1 % |
| (b) Carbendazim (Bavistin 50 WP): | : | 0.05 % |
| (c) PMA (PMA 1 %) | : | 0.001 % |
| (d) Carbofuran (Furadan 3 G) | : | 50 g/tree |
| (e) B.M. + Carbofuran | : | 1 % + 50 g |
| (f) Carbendazim + Carbofuran | : | 0.05 % + 50 g |
| (g) PMA + Carbofuran | : | 0.001 % + 50 g |
| (h) Control (water) | : | - |

(4) Replications : Three

(5) Variety : Deshi

3.9 DISEASE INDEX :

The incidence of disease was recorded after 45 days of last drenching. Thirty twigs were selected randomly from each tree of mango and graded into five classes (0 - 4 scale) based on intensity of the disease.

3.10 DISEASE RATING SCALE :

For comparing disease condition in different trees of same variety (Deshi) of mango an arbitrary scale with numerical rating was proposed.

Class	Description
0	Twig completely healthy.
1	Disease present only near the tip of the twig with a little browning of apical portion and leaves completely healthy.
2	Twig bearing partially diseased leaves.
3	All the leaves at the top of the branch completely dried.
4	The branch completely dead and defoliated with exudation of gum.

The disease index was worked out by using the following formula :

$$\text{Disease index} = \frac{\text{Sum of numerical value}}{\text{No. of twigs observed} \times 4} \times 100$$

Per cent reduction of disease intensity in each treatment over control was worked out by using following formula.

$$\text{Per cent reduction of disease intensity} = \frac{\text{Disease in treatment} - \text{Average disease in control}}{\text{Average disease in control}} \times 100$$

Statistical analysis was carried out following Panse and Sukhatme (1967).

**RESULTS
&
DISCUSSION**

CHAPTER - IV

RESULTS AND DISCUSSION

4.1 SYMPTOMATOLOGY :

The initiation of symptoms are seen at the tips of leaves. Gradually infection advances on both the margins of the leaves. The area around the midrib remains green, then infection spreads on entire leaf covering most of the areas of the leaf. Thus within short period of time, marginal blighting is quite commonly seen on the infected twigs and it appears as scorched by fire (Plate 1). Green tender growth of the twigs/branches after infection, turn deep brown to blackish in colour. It advances downward along the twigs/branches. The infected brownish twigs/branches are shrivelled, dried and develop cracking on bark. From the healthy portion below the node, new shoots emerge out in a whirl around the node, however, the leaves remain smaller in size and get infected even at brownish tender stage. Thus, when the brown tender new growth get infected, it withers out within a short period. Near the axil of the healthy area, but in the dried blackish portion of the branch gummy exudation is quite commonly seen (Plates 2A & 2B). The affected tree shows varying degree of leaf blighting, tip withering, defoliated twigs/branches, accompanied by brownish discolouration, shrivelling and death. Majority of twigs/branches and the basal trunk develop bark canker and produce gummy exudation, eventually leading to die-back situation of the entire tree (Plate 3).

Plate 1 : Initial symptoms of mango
twig blight.



Plate 2A : Diseased mango twig showing gum exudation.



Plate 2B : Gum exudation leading to twig distortion

Generally in diseased tree with leaf blighting, twig blight, die back, gumming type symptoms in advance stage and even degeneration of entire tree may also be seen (Plate 3). Thus the symptoms noticed during the present investigations are in conformity with the reports of Verma and Singh (1970); Rath et al. (1978); Rodriguez Torres (1978); Pathak (1980); Chattopadhyay et al. (1982); Davis et al. (1987).

4.2 MORPHOLOGICAL CHARACTERS :

In laboratory, isolations were carried out from various diseased parts of the tree i.e. leaf, twigs/ branch and gummy bark. This was purified and ultimately sent for proper identification at IMI, kew, Surry, England. It was identified as Botryodiplodia theobromae Pat. The description of the colony characters, hyphae, fruiting body and spores are on the basis of fungal growth on PDA at room temperature ($28^{\circ} \pm 1^{\circ} \text{C}$).

Colony :

On potato-dextrose-agar fluffy, cottony white growth was observed initially which covered the petridish within two to three days. The growth was depressed in the centre whereas it was fluffy and raised in the rest of the area which touched the lid of the petridish. After two to three days colour of the colony changed olivaceous to dark, while completely blackish background was noticed at the lower surface of petridish.

Plate 3 : Tree showing advanced symptoms
of the disease.



Mycelium :

Initially the hyphae of the mycelium were hyaline, aseptate, which later turned to septate and dark brown, on potato-dextrose-agar (Plate 4).

Pycnidial bodies :

On potato-dextrose-agar, few black pycnidial bodies were observed on upper surface after ten days of growth. Brown to black pycnidial bodies were also noticed when petridishes were kept against the light which were confirmed by microscopic examination. Pycnidial bodies were subglobose to globose, erumpent and ostiolated. Size of pycnidia were 145.0 to 396.4 μm X 107.0 to 323.5 μm with an average of 289.74 X 219.13 μm .

Pycnidiospores :

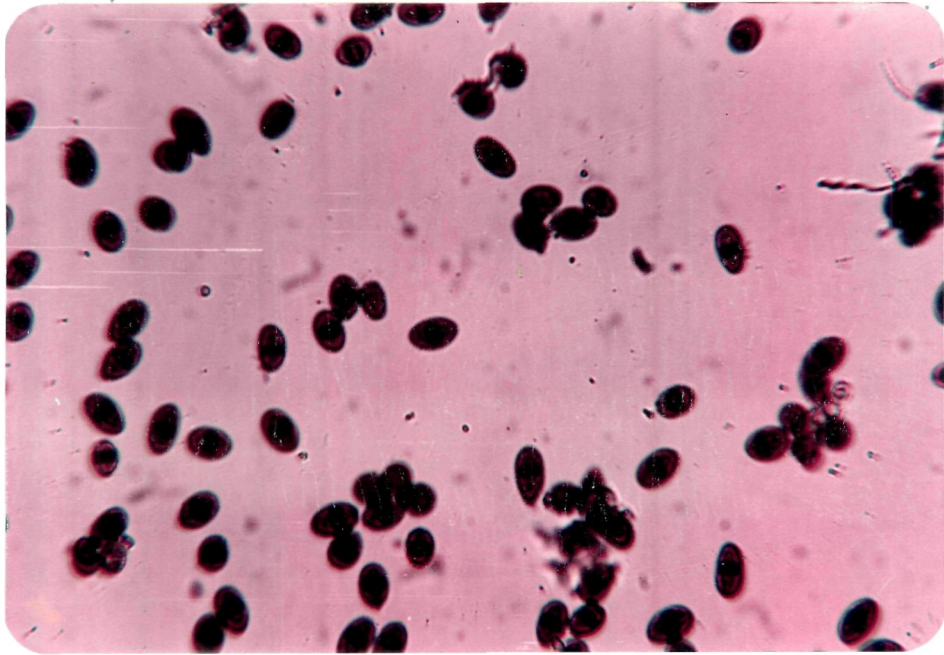
The pycnidiospores oozed out were initially immature, hyaline, thin walled, globose to subglobose and single celled with bluish dots in the cells. Later the spores became bicelled dark brown thick walled, doliform to shortly cylindrical at maturity (Plates 5A & 5B). Average size of pycnidiospores was 26.4 X 11.3 μm . Reisolation of the organism from diseased parts yielded exactly similar type of pure culture and under microscopic observations it was found identical to that of Botryodiplodia theobromae producing initially hyaline, one celled, thin walled, globose to subglobose pycnidiospores. Later turning into brownish black, two celled, thick walled spores with longitudinal striations (Plate 5B).

Plate 4 : Septate mycelium with pycnidiospores.



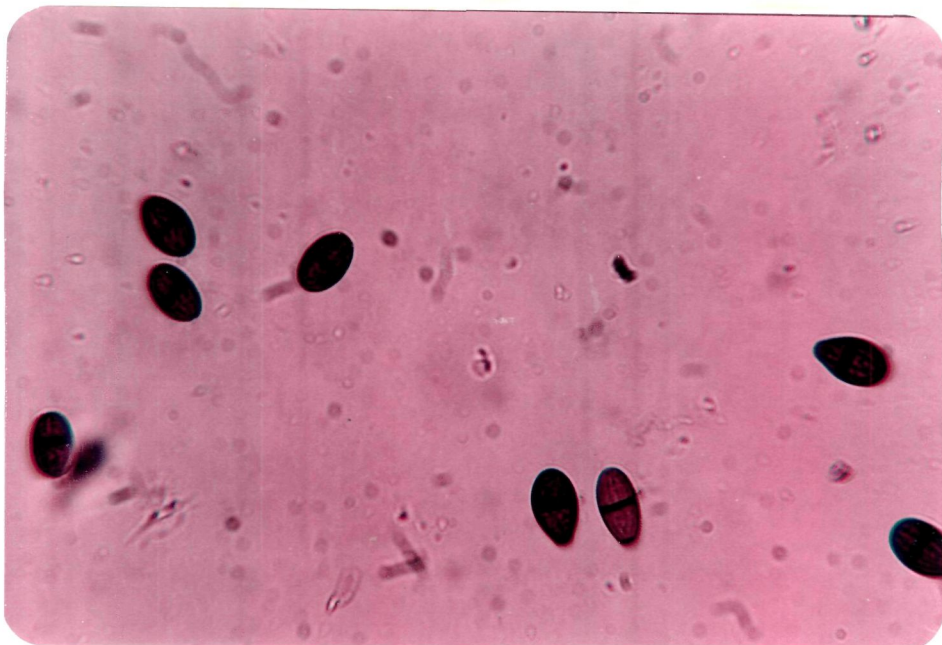
(25x)

Plate 5A : Pycnidiospores (25 X).



(25x)

Plate 5B : Pycnidiospores (40 X).



(40x)

These characteristics are similar as Botryodiplodia theobromae Pat. earlier known to be Diplodia natalensis. The general characteristics of fungus in respect of morphology i.e. colony, size of the pycnidia and the pycnidiospores are found similar to those reported by Chona (1933); Alasoadura (1970); Patel (1971); Pathak (1980); Chattopadhyay et al. (1982); Prabhakara Rao and Deshmukh (1986) and Davis et al. (1987).

4.3 PATHOGENICITY :

4.3.1 On mango seedlings :

After three to four days of inoculation, brownish dark dots were observed at the point of inoculation. Thereafter black, necrotic area gradually enlarged downward and also towards the tip of seedlings. After 12 days of inoculation, there was oozing of yellowish fluid from infected area which remained on the surface in form of droplets. Later on there was a discolouration, shrivelling and drying of entire seedlings (Plates 6A & 6B).

4.3.2 On fruits :

After three to four days of fruit inoculation slight darkening was observed at the point of inoculation. The affected area enlarged and showed dark brown to black colour. Infection also advanced to form a big, dark, circular patch. Later the whole fruit turned completely black and then deformed. The pulp became lightbrown

Plate 6A : Mango seedlings - Left inoculated.



Plate 6B : Mango seedling - Inoculated with B. theobromae (Late stage).



and soft. The fruit symptoms noticed are in accordance with the earlier reports made by several workers (Uppal et al., 1934; Lele et al., 1975; Rath and Mohanan, 1977; Bārhan, 1987 and Lutchmeah, 1988).

4.4 EFFECT OF LIQUID MEDIA ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT. :

Nine different synthetic and non-synthetic liquid media were tried to find out a suitable medium for maximum growth and sporulation of the fungus. Observations recorded on growth in form of dry mycelial weight and pycnidial production after 15 days of incubation period are presented in Table 1 and Fig. 1.

It is revealed from the results that the dry mycelial weight was significantly different on each medium. Amongst all, Richard's medium supported maximum mycelial growth (763.00 mg). Czapek's medium was found next best (456.50 mg) to Richard's medium, followed by potato dextrose broth (201.50 mg). Asthana and Hawker's solution supported poor growth (68.50 mg).

Considering liquid media, Richard's medium was the best for sporulation. The next best was Czapek's medium, while Asthana and Hawker's medium did not help to produce spores. Richard's medium was reported to be better for growth and sporulation of Botryodiplodia theobromae Pat. by Siradhana (1960^g); Verma (1968) and Patel (1971).

Table 1. Effect of different liquid media on the growth of B. theobromae Pat.

Sr. No.	Media	Dry mycelial* wt.(mg)	Pycnidial** production	Final pH
1.	Asthana and Hawker's medium	1.8298 (68.50)	-	8.0
2.	Brown's medium	2.2963 (198.25)	+	7.8
3.	Czapek's medium	2.6575 (456.50)	+	7.9
4.	Elliot's medium	1.9026 (81.00)	+	8.1
5.	Modified Asthana and Hawker's medium	1.8234 (69.75)	-	7.9
6.	Potato Dextrose Broth	2.3024 (201.50)	+	8.0
7.	Potato Sucrose Broth	2.2346 (172.00)	+	8.5
8.	Richard's medium	2.8822 (763.00)	+	7.6
9.	Standard nutrient medium	2.2036 (160.25)	+	7.8

S.Em. : 0.036

C.D. 0.05 : 0.106

C.V.% : 3.252

* Average of four replications

** Pycnidial production present : +

Pycnidial production absent : -

Figures in the parentheses indicate retransformed value.

- AH = Asthana and Hawker's medium
- BM = Brown's medium
- CM = Czapek's medium
- EM = Elliot's medium
- MAH = Modified Asthana and Hawker's medium
- PDB = Potato dextrose broth
- PSB = Potato sucrose broth
- RM = Richard's medium
- SNM = Standard nutrient medium

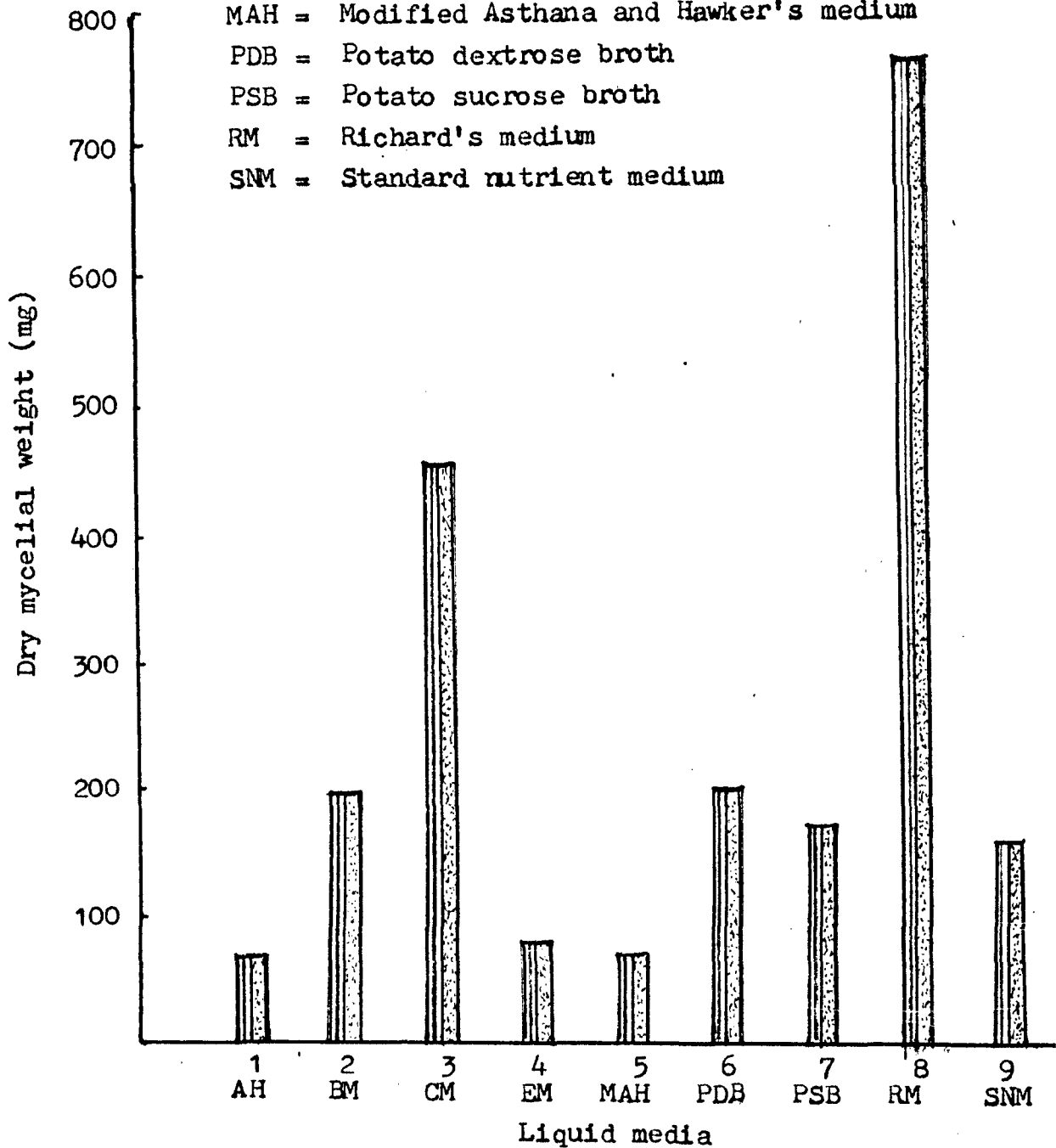


Fig. 1 : Effect of different liquid media on the growth of B. theobromae Pat.

Similarly Czapek's medium was also found good as reported by Bhatnagar (1970) and Patel (1971). Asthana and Hawker's medium was reported to be poor source by Verma (1968) and Bhatnagar(1970). Thus results are in line with the others.

Richard's medium and Czapek's medium contain potassium phosphate, magnesium sulphate and sucrose. One of them must have contributed for better growth. Asthana and Hawker's medium, containing potassium nitrate, magnesium sulphate and glucose, proved to be better sources in the physiological study regarding nitrogen and carbon sources, respectively; hence the same can not be responsible for inhibiting the growth of the fungus. Therefore, magnesium sulphate may be responsible for reducing the growth of fungus. Richard's medium was found to be the best for growth and sporulation and hence was selected for further physiological studies of the fungus.

4.5 EFFECT OF INCUBATION PERIOD ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT. :

The growth rate of Botryodiplodia theobromae increased (from 255.50 to 308.00 mg dry mycelial weight) significantly up to 15 days of incubation and thereafter decreased on 20th, 25th, 30th and 35th day of incubation. In present investigation mycelial growth was maximum on 15th day of incubation and was selected for further physiological studies. The data are presented in Table 2 and Fig. 2.

Table 2. Effect of ⁱIncubation period on the growth of B. theobromae Pat.

Sr. No.	Incubation period (days)	Dry mycelial* weight (mg)	Pycnidial** production	Final pH
1.	5	264.50	-	7.0
2.	10	275.00	-	7.3
3.	15	308.00	+	8.2
4.	20	290.25	+	7.8
5.	25	284.25	+	8.0
6.	30	264.75	+	7.9
7.	35	255.50	+	8.3

S.Em. : 4.257

C.D. 0.05 : 12.521

C.V.% : 3.069

* Average of four replications.

** Pycnidial production present : +

Pycnidial production absent : -

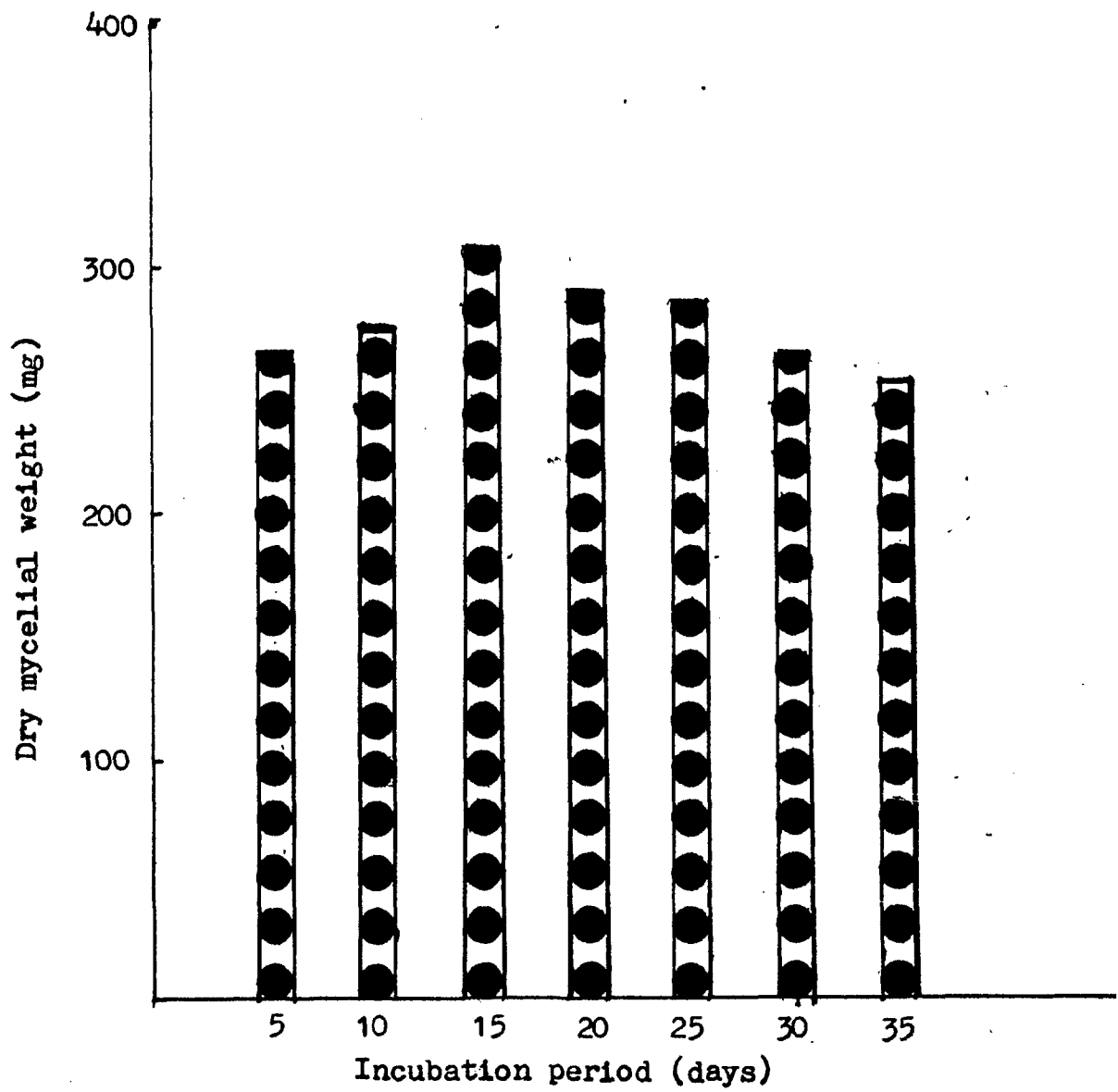


Fig. 2 : Effect of incubation period on the growth of *B. theobromae* Pat.

4.6 EFFECT OF TEMPERATURE ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT. :

To determine the optimum temperature for the growth and sporulation, the fungus was grown at different temperatures varying from 5° to 40°C. Observations on the dry mycelial weight and pycnidial production recorded after 15 days of incubation are presented in Table 3 and Fig. 3.

The fungus grew well between temperature range of 20° to 40°C and sporulated in the range of 20° to 35°C. Dry mycelial weight was significantly better at 30°C (939.25 mg) than the rest, except at 25°C (921.25 mg) which is at par. Both were significantly better than the rest. Therefore, the optimum temperature is between 25° to 30°C for both growth and sporulation. The optimum temperature range observed during the present studies i.e. 25° - 30°C is in agreement with those reported by Verma and Singh (1970) on mango; Tsai (1983); Seehann and Tabirih (1983) and Mattos and Ames (1986). Some of the workers also reported that the temperature around 30°C to be better for the growth of Botryodiplodia theobromae Pat; (Siradhana, 1960^b; Srivastava and Tandon, 1963; Patel, 1971; Ogundana et al., 1971; Pathak, 1980; Nwifo and Fajola, 1986 and Mali and Bhatnagar, 1987^a).

4.7 EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT. :

Dry mycelial output, sporulation and final pH observed after 15 days due to effect of various initial

Table 3. Effect of various levels of Temperature on the growth of B. theobromae Pat.

Sr. No.	Temperature (°C)	Dry mycelial wt.(mg)	Pycnidial** production
1.	5	-	-
2.	10	42.00	-
3.	15	94.50	-
4.	20	408.75	+
5.	25	921.25	+
6.	30	939.25	+
7.	35	455.25	+
8.	40	93.00	-

S.Em. : 6.199

C.D. 0.05 : 18.235

C.V.% : 2.938

* Average of four replications.

** Pycnidial production present : +

Pycnidial production absent : -

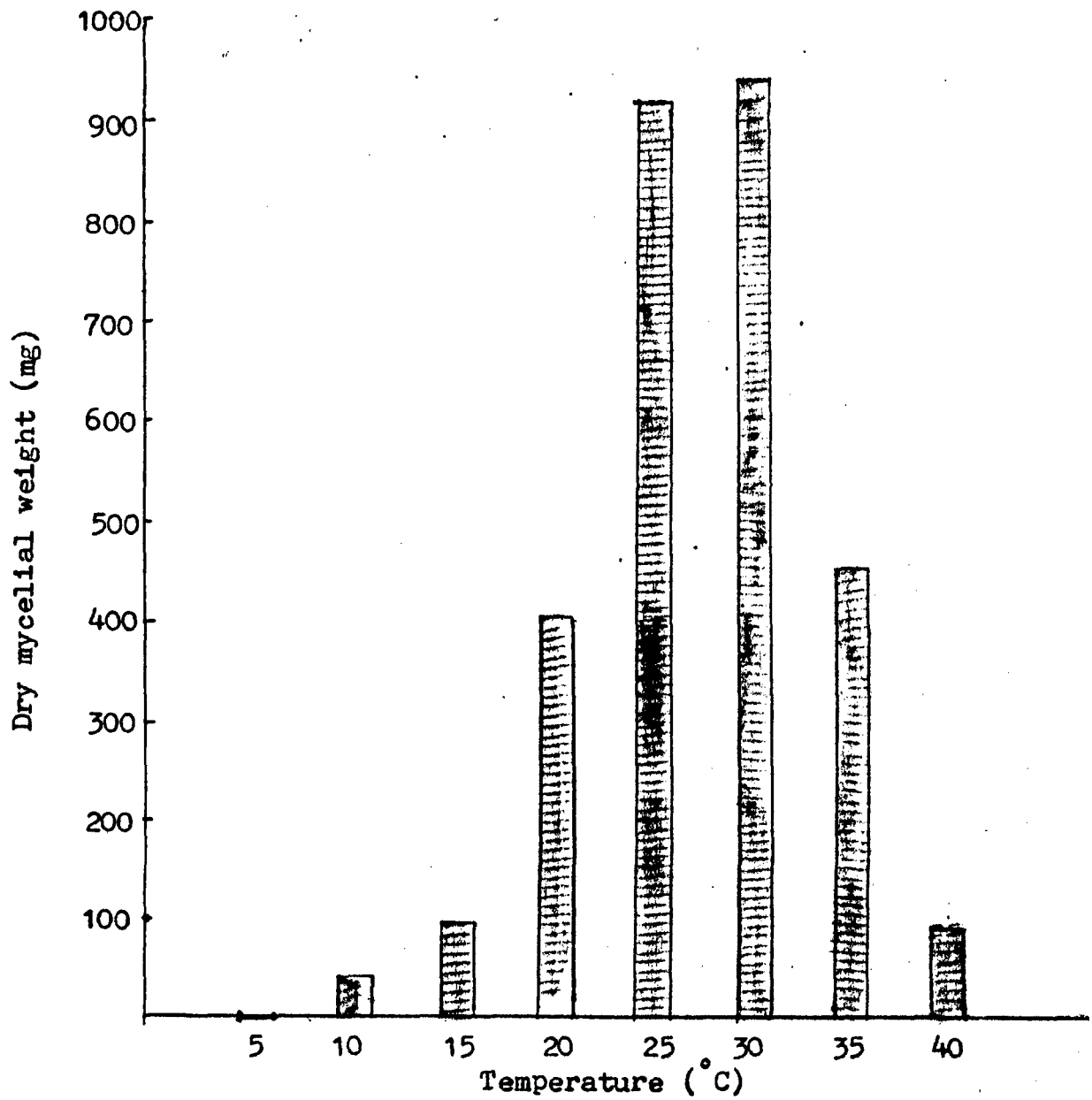


Fig. 3 : Effect of temperature on the growth of B. theobromae Pat.

levels of pH in liquid media, are presented in Table 4 and Fig. 4. Dry mycelial weight indicated that fungus can grow in a wide range of pH 4.0 to 9.0; however, sporulation occurred between pH 5.5 to 7.0. Yield of dry mycelial growth was significantly better at pH 6.0 (510.50 mg) followed by 6.5 (501.50 mg), while sporulation at pH 6.5 was better, followed by pH 6.0.

Thus slightly acidic condition is more favourable as compared to alkaline medium. In any case final pH of the media was alkaline in nature. Verma (1968); Srivastava and Tandon (1968) and Patel (1971) reported that pH 6.0 to 6.5 was better for growth and sporulation of Botryodiplodia theobromae Pat. These results are very closely in accordance with the results obtained during the present studies.

4.8 EFFECT OF DIFFERENT CARBON SOURCES ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT. :

The effect of different carbon sources on the growth and production of pycnidia of Botryodiplodia theobromae Pat. was compared in basal medium i.e. Richard's medium. After 15 days of incubation period, output of the dry mycelial mat and sporulation are presented in Table 5 and Fig. 5. It is evident that carbon is an essential nutrient for good growth of the fungus as the media enriched with carbon gave good growth of the fungus (Table 5). Fungus grew well on all carbon sources, however, variation in the growth was observed.

Table 4. Effect of hydrogen-ion concentration on the growth of B. theobromae Pat.

Sr. No.	pH	Dry mycelial wt. (mg) [*]	Pycnidial production ^{**}	Final pH
1.	4.0	206.25	-	8.1
2.	4.5	232.25	-	8.8
3.	5.0	338.00	-	8.7
4.	5.5	426.50	+	8.8
5.	6.0	510.50	+	8.5
6.	6.5	501.50	+	8.5
7.	7.0	401.75	+	8.6
8.	7.5	393.50	+	7.8
9.	8.0	376.75	-	7.9
10.	8.5	271.50	-	7.7
11.	9.0	239.25	-	8.0

S.Em. : 5.053

C.D. 0.05 : 14.593

C.V.% : 2.852

* Average of four replications.

** Pycnidial production present : +
Pycnidial production absent : -

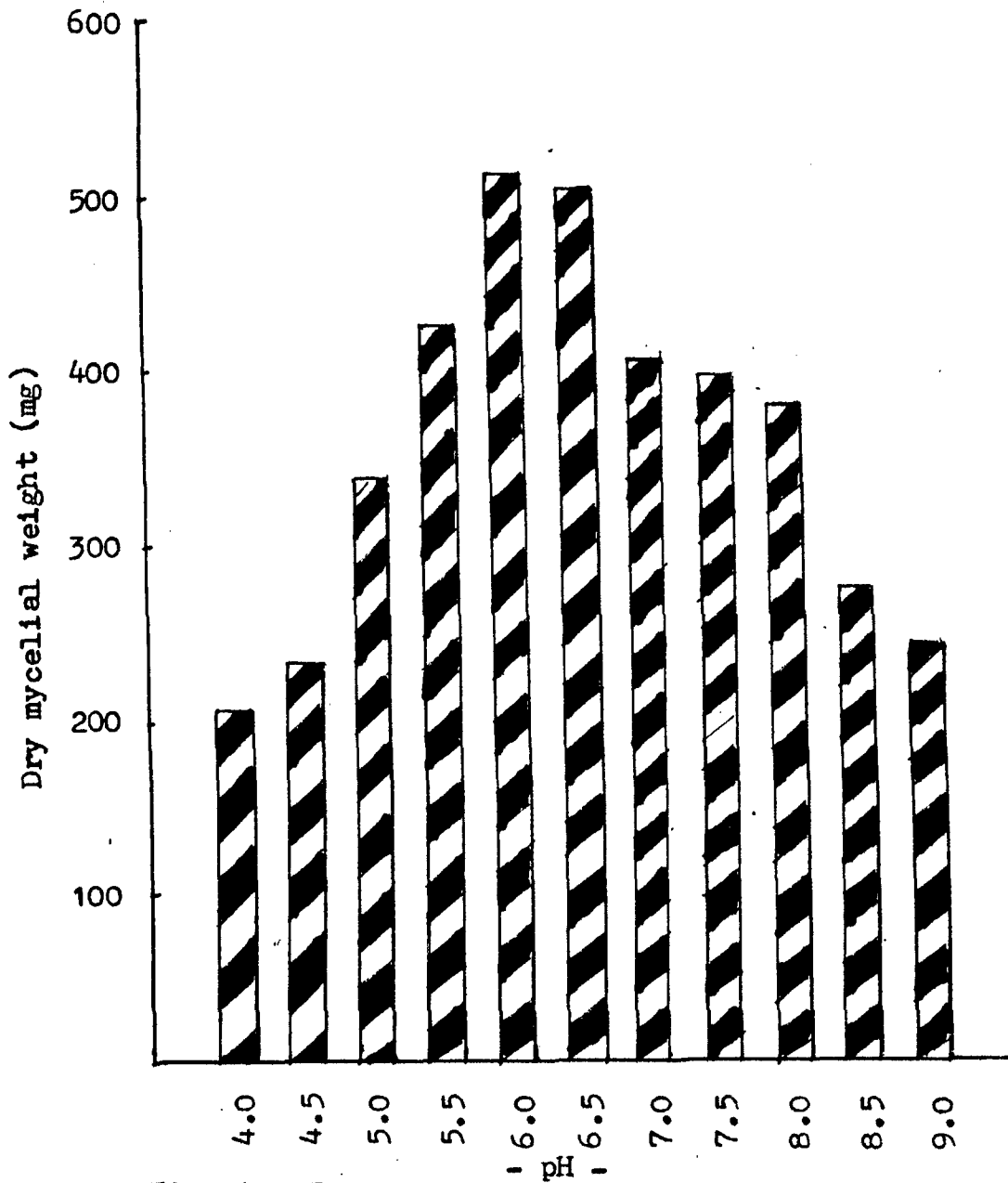


Fig. 4 : Effect of hydrogen-ion concentration on the growth of *B. theobromae* Pat.

Table 5. Effect of different carbon sources on the growth of B. theobromae Pat.

Sr. No.	Carbon sources	Dry mycelial* wt. (mg)	Pycnidial** production
1.	Fructose	405.50	+
2.	Galactose	86.25	+
3.	Glucose	620.25	+
4.	Lactose	224.75	+
5.	Maltose	587.50	+
6.	Starch	46.50	+
7.	Sucrose	580.50	+
8.	Xylose	118.75	+
9.	Control (No carbon source)	20.50	-

S.Em. : 5.366

C.D. 0.05 : 15.572

C.V.% : 3.590

* Average of four replications.

** Pycnidial production present : +

Pycnidial production absent : -

FR = Fructose ST = Starch
 GA = Galactose SU = Sucrose
 GL = Glucose XY = Xylose
 LA = Lactose C = Control
 MA = Maltose (Without carbon)

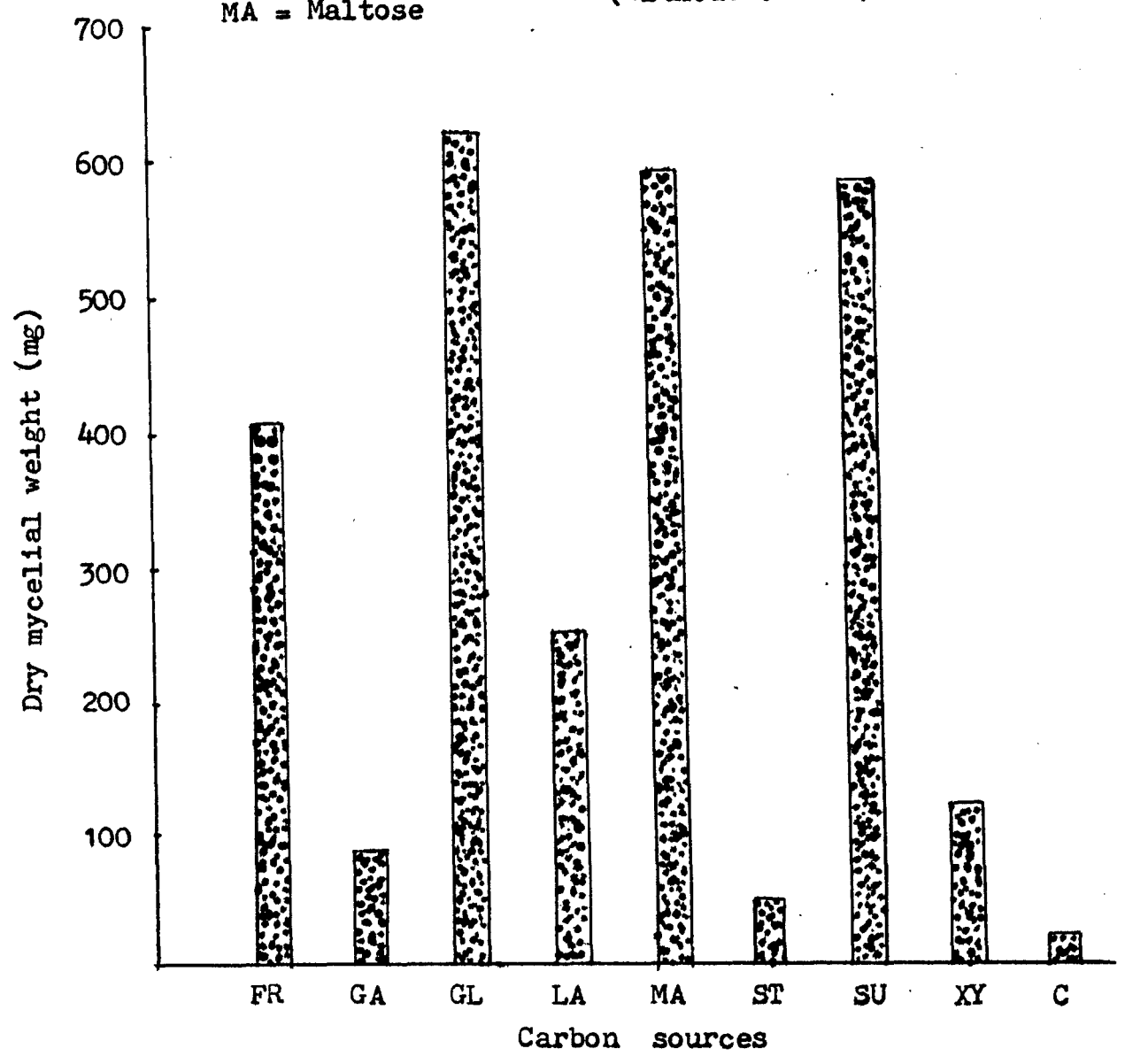


Fig. 5 : Effect of carbon sources on the growth of B. theobromae Pat.

Considering the dry mycelial weight, glucose (620.25 mg), maltose (587.50 mg), sucrose (580.50 mg) and fructose (405.50 mg) were significantly better over other sources of carbon. Starch (46.50 mg) was significantly poor source among the carbon sources tested.

Similar results of good growth of B. theobromae on glucose was observed by Margolin (1940); Bhatnagar (1970); Verma and Prasad (1975); Seehann and Tabirih (1983) and Kaul (1987). Sucrose was reported to be better source of carbon by Margolin (1940) and Prasad (1965). 'Maltose', 'Mannose' and 'Sucrose' were reported to be good sources (Mali and Bhatnagar, 1987^b). 'Glucose', 'Sucrose' and 'Maltose' (Patel, 1971 and Ahmed and Haq, 1973) and 'Lactose' have been reported as poor source (Margolin, 1940).

4.9 EFFECT OF DIFFERENT NITROGEN SOURCES ON THE GROWTH OF Botryodiplodia theobromae Pat. :

The effect of different nitrogen source on growth and sporulation of B. theobromae Pat. was compared in the Richard's medium. Observations recorded on dry weight of the mycelial mat and sporulation after 15 days of incubation period are presented in Table 6 and Fig. 6.

Considering the various sources of nitrogen 'Calcium nitrate' supported highest growth (673.25 mg) compared to others while next best source was 'Potassium nitrate' (586.00 mg) followed by 'Urea' (525.00 mg). In general nitrate sources were better as compared to ammonical

Table 6. Effect of different nitrogen sources on the growth of B. theobromae Pat.

Sr. No.	Nitrogen sources	Dry mycelial* wt. (mg)	Pycnidial** production
1.	Ammonium acetate	464.25	+
2.	Ammonium nitrate	525.00	+
3.	Ammonium oxalate	454.00	+
4.	Ammonium phosphate	285.25	+
5.	Ammonium sulphate	222.25	+
6.	Calcium nitrate	673.25	+
7.	Potassium nitrate	586.00	+
8.	Sodium nitrate	487.75	+
9.	Urea	525.00	+
10.	Control (No nitrogen source)	118.50	-

S.Em. : 4.073

C.D. 0.05 : 11.762

C.V.% : 2.000

* Average of four replications.

** Pycnidial production present : +

Pycnidial production absent : -

AA = Ammonium acetate	CN = Calcium nitrate
AN = Ammonium nitrate	PN = Potassium nitrate
AO = Ammonium oxalate	SN = Sodium nitrate
AP = Ammonium phosphate	U = Urea
AS = Ammonium sulphate	C = Control (Without nitrogen)

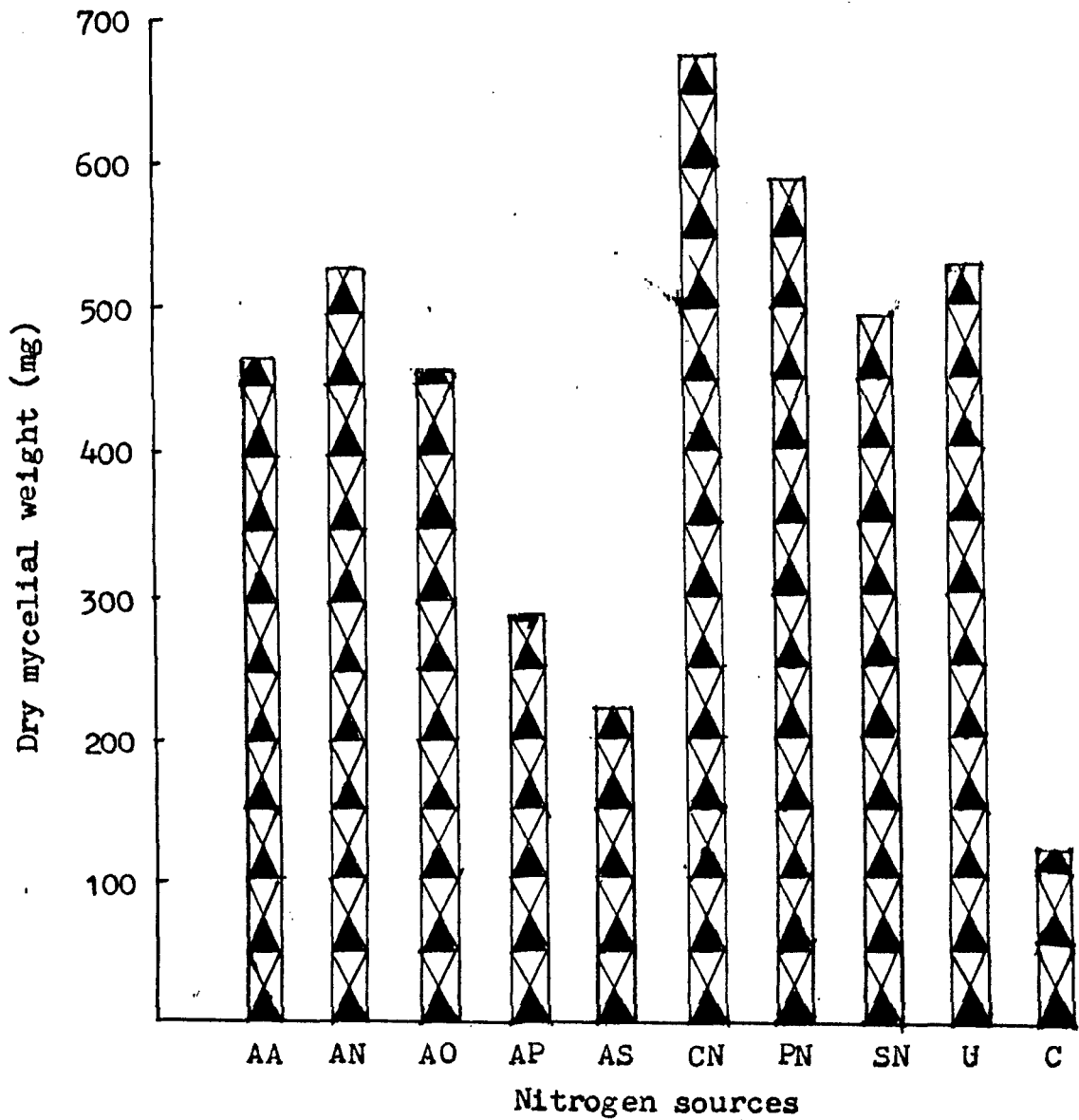


Fig. 6 : Effect of nitrogen sources on the growth of B. theobromae Pat.

sources. The nitrogen source in the form of 'Ammonium sulphate' was significantly better than no nitrogen (control), however, the same proved to be most inferior source among all the sources tested.

As against the normal source 'Potassium nitrate', 'Calcium nitrate' was significantly better than the rest of sources and same was also reported as good source by Prasad(1965); Patel(1971); Tsai(1983). Nwifo and Fajola (1986) also reported 'Potassium nitrate' and 'Sodium nitrate' as good sources of nitrogen. Kaul(1987) also reported 'Calcium nitrate' a better source. From the results obtained it is clear that the nitrate sources were definitely better for the growth of B. theobromae, a pathogen causing disease in many fruit crops. The poor growth of the fungus in the medium devoid of any nitrogen source indicated that it is an essential nutrient for the growth of the fungus(Table 6).

Since nitrate sources were better as compared to ammonical sources of nitrogen and 'Ammonium sulphate' yielded the poorest growth of the fungus, it would be interesting to observe the possible effects of different kinds of nitrogenous fertilizers on the incidence of the disease.

4.10 EFFECT OF DIFFERENT COMBINATION OF 'CARBON AND NITROGEN' SOURCES ON THE GROWTH OF BOTRYODIPLODIA THEOBROMAE PAT.

Dry mycelial output and sporulation on various combinations of 'Carbon' and 'Nitrogen' sources are presented in Table 7 and Fig. 7.

Table 7. Effect of different combinations of carbon and nitrogen sources on the growth of B. theobromae Pat.

Sr. No.	Combinations of carbon and nitrogen	Dry mycelial* wt. (mg)	Pycnidial** production
1.	Calcium nitrate X Glucose	278.00	+
2.	Calcium nitrate X Maltose	602.00	+
3.	Calcium nitrate X Sucrose	586.75	+
4.	Potassium nitrate X Glucose	515.50	+
5.	Potassium nitrate X Maltose	449.00	+
6.	Potassium nitrate X Sucrose	385.00	+
7.	Urea X Glucose	476.25	+
8.	Urea X Maltose	345.00	+
9.	Urea X Sucrose	436.25	+

S.Em. : 3.809

C.D. 0.05 : 11.055

C.V.% : 1.683

* Average of four replications.

** Pycnidial production present : +

Pycnidial production absent : -

CN.G = Calcium nitrate X Glucose
 CN.M = Calcium nitrate X Maltose
 CN.S = Calcium nitrate X Sucrose
 PN.G = Potassium nitrate X Glucose
 PN.M = Potassium nitrate X Maltose
 PN.S = Potassium nitrate X Sucrose
 U.G = Urea X Glucose
 U.M = Urea X Maltose
 U.S = Urea X Sucrose

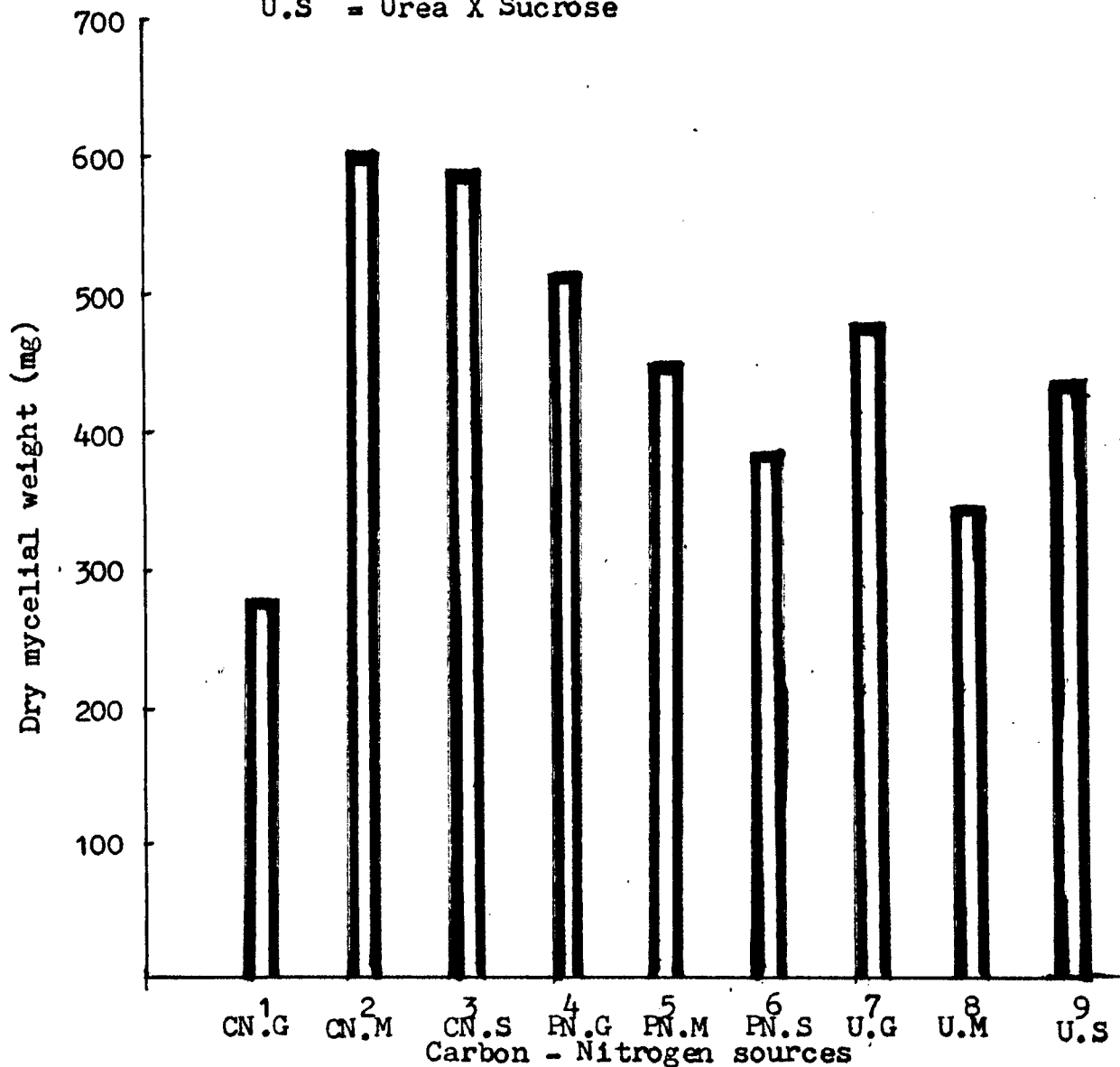


Fig. 7 : Effect of different combinations of carbon and nitrogen sources on the growth of B. theobromae Pat.

Disaccharide 'maltose' and 'sucrose' both were found significantly better in combination with 'calcium nitrate' with a maximum mycelial output (602.00 mg) and (586.75 mg) respectively, over the rest of combinations. While monosaccharide 'glucose' in combination with 'calcium nitrate' was the poorest (278.00 mg) among the various combinations tried. The next best combination was 'potassium nitrate' with monosaccharide 'glucose' (515.50 mg) which was significantly better than the rest of other combinations. 'Urea' in combination with monosaccharide 'glucose' yielded better (476.25 mg) as compared to disaccharides. Taking into consideration both dry weight of mycelium and sporulation, combination of 'calcium nitrate' and 'maltose' appeared to be the best.

Sources of carbon (glucose, maltose and sucrose) and nitrogen (calcium nitrate, potassium nitrate and urea) were tested in which 'calcium nitrate' and 'maltose' combination proved to be almost significantly better in production of dry mycelial weight and sporulation as compared to rest of the combinations. Other better combinations were 'calcium nitrate' and 'sucrose' and 'potassium nitrate' and 'glucose'.

For obtaining good growth, it is necessary to replace 'calcium nitrate', in place of 'potassium nitrate' and 'maltose' in place of 'sucrose' in Richard's medium.

It has already indicated that individual sources of carbon and nitrogen have greater importance and the appropriate combinations have even more significant role in obtaining better growth and sporulation.

4.11 EVALUATION OF SYSTEMIC AND NON-SYSTEMIC FUNGICIDES AGAINST BOTRYODIPLDIA THEOBROMAE PAT. IN VITRO :

Twelve systemic and non-systemic fungicides were assayed in the laboratory by the 'paper disc method' and data are presented in Table 8. The data (Table 8) indicated that out of twelve different systemic and non-systemic fungicides, only ten fungicides were effective in checking the growth of the fungus. The other two viz., carboxin (Vitavax 75 WP) and bordeaux mixture failed to check the growth of fungus even at higher concentration (4000 ppm). Similarly, all the concentrations of each fungicide differed significantly in respect of inhibition zone.

Among ten effective fungicides, carbendazim (Bavistin 50 WP) showed maximum inhibition zone even at a concentration of 250 ppm and above followed by MEMC 6 %, FMA 1 %, mancozeb (Dithane M-45 75 WP) at 1000 ppm and above (Plates 7A & 7B). While, captafol (Difolatan 80 WP); thiophenate methyl (Topsin M 70 % WP), captan (Captaf) and aureofungin were more or less effective at 2000 ppm and above concentrations. Triforin (Bapro) and

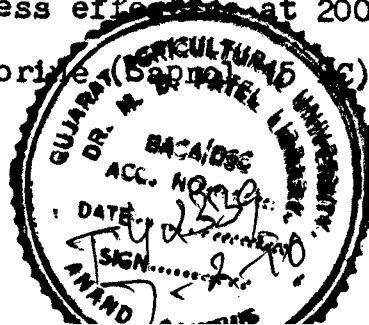


Plate 7A



Plate 7A & 7B : In-vitro evaluation of fungicides against B. theobromae by paperdisc method.

Plate 7B



Table 8. Evaluation of systemic and non-systemic fungicides against B. theobromae Pat. in vitro.

Sr. No.	Fungicides	Inhibition zone (mm)*				
		250 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm
1.	Carbendazim	15.12	21.69	27.44	28.14	30.51
2.	Mancozeb	0.00	0.00	18.30	21.63	26.59
3.	FMA 1 %	0.00	0.00	16.39	19.15	23.36
4.	MEMC 6 %	0.00	0.00	21.38	22.98	26.56
5.	Captafol	0.00	0.00	0.00	14.31	16.48
6.	Thiophanate methyl	0.00	0.00	0.00	15.37	18.34
7.	Aureofungin	0.00	0.00	0.00	15.36	16.23
8.	Thiram	0.00	0.00	0.00	0.00	14.28
9.	Tri-farine	0.00	0.00	0.00	0.00	9.17
10.	Captan	0.00	0.00	0.00	10.08	11.35
11.	Carboxin	0.00	0.00	0.00	0.00	0.00
12.	B.M.	0.00	0.00	0.00	0.00	0.00

Source	S.Em.	C.D. 0.05	C.V.%
Treatment	0.104	0.287	
Concentration	0.073	0.203	5.021
Treatment X Concentration	0.232	0.643	

* Average of four replications.

- | | |
|-----------------|------------------------|
| 1 = Carbendazim | 6 = Thiophanate methyl |
| 2 = Mancozeb | 7 = Aureofungin |
| 3 = PMA 1 % | 8 = Thiram |
| 4 = MEMC 6 % | 9 = Triforine |
| 5 = Captafol | 10 = Captan |

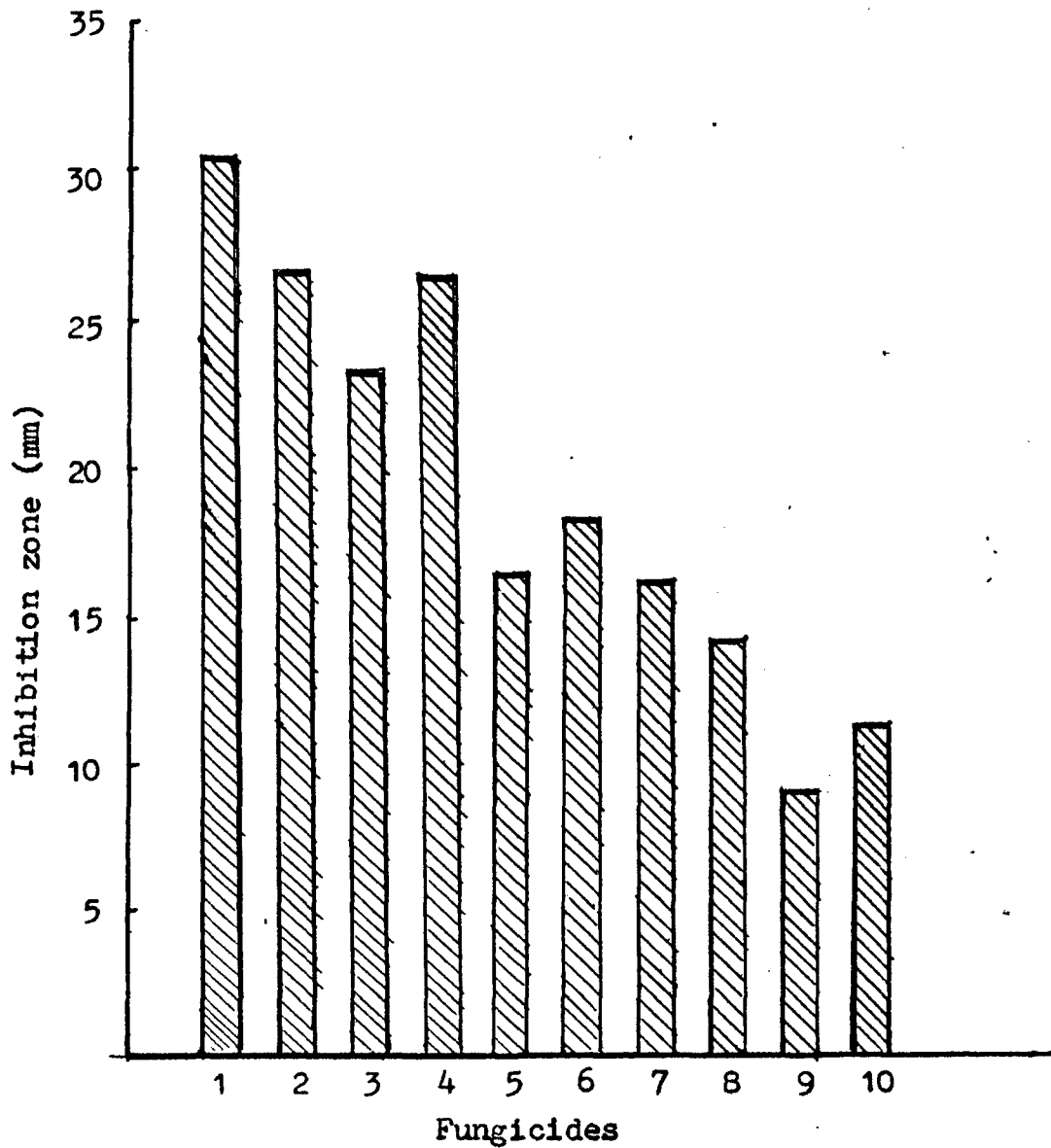


Fig. 8 : Effect of various fungicides at 4000 ppm concentration against B. theobromae Pat.

thiram (Danatax) were effective only at 4000 ppm among the various concentrations tried during the present investigation.

Among various fungicides evaluated, carbendazim (Bavistin) was most effective in checking the growth of the fungus even at the lowest concentration (250 ppm). The results obtained during the present studies are in agreement with those reported by Bammi et al. (1972); Sohi et al. (1973); Vyas and Singh (1977); Kumbhare and Chaudhari (1979); Anusuya (1984) and Utikar et al. (1986). Akanda and Fakir (1985); Ogawa et al. (1968) and Ogundana and Dennis (1981) reported that the mancozeb (Dithane M-45) and captan (Captaf) were effective against B. theobromae Pat. Similar reports were also made by Srivastava and Tandon (1971) and Utikar et al. (1986). Raghvan and Saksena (1978) reported captan (Captaf) to be effective against B. theobromae. Adisa and Fajola (1982) reported captan (Captaf) and captafol (Difolatan) to be effective against B. theobromae Pat., in vitro. Pandey et al. (1980) and Bhargava et al. (1983) reported that triforine (Saprol 15 EC) gave good control of B. theobromae Pat. on banana. Captafol (Difolatan) and carbendazim (Bavistin) were effective against B. theobromae Pat. (Anusuya, 1984). Raghvan et al. (1979); Agarwal et al. (1982) and Atri (1985) reported that aureofungin was effective against B. theobromae Pat. in vitro.

4.12 EFFICACY OF VARIOUS CHEMICALS AGAINST BOTRYODIPLODIA
THEOBROMAE PAT. IN ORCHARD :

A field trial was conducted at Mango orchard, B.A. College of Agriculture, Anand with a view to select a suitable fungicide for the control of Botryodiplodia theobromae Pat. Chemicals included in the trial were carbendazim (Bavistin 50 WP); PMA 1 %, carbofuran (Furadan 3G), bordeaux mixture (B.M.) and combinations viz., carbendazim + carbofuran, B.M. + carbofuran, PMA + carbofuran. Results on mean disease index and per cent reduction over control are presented in Table 9.

It is evident from the data (Table 9) that all the chemicals were significantly effective in reducing the disease as compared to check. None of the chemicals gave complete control of the disease. However, carbendazim was significantly superior over all other treatments. Carbendazim + carbofuran and PMA 1 % were next in order. PMA + carbofuran, bordeaux mixture, carbofuran and B.M. + carbofuran were least effective. Thus the order of efficacy of chemicals being carbendazim (Bavistin), carbendazim + carbofuran, PMA 1 %, PMA + carbofuran, bordeaux mixture, carbofuran (Furadan 3G) and B. M. + carbofuran.

Table 9. Effect of different chemicals on intensity of disease caused by B. theobromae Pat. in field/orchard.

Sr. No.	Treatment	Concentration	Mean* Disease Index(%)	Per cent reduction over control
1.	Bordeaux mixture	1 %	71.70	13.96
2.	Carbendazim	0.05 %	41.39	50.33
3.	FMA 1 %	0.001 %	62.50	24.99
4.	Carbofuran	50 g	74.72	10.33
5.	B.M. + Carbofuran	1 % + 50g	75.00	9.99
6.	Carbendazim + Carbofuran	0.05 % + 50 g	55.00	33.99
7.	FMA + Carbofuran	0.001 % + 50 g	69.17	16.99
8.	Control (Water)	-	83.33	-

S.Em. : 1.742

C.D. 0.05 : 5.284

C.V.% : 3.780

* Average of three replications.

**SUMMARY
&
CONCLUSIONS**

CHAPTER - V

SUMMARY AND CONCLUSIONS

Twig blight with gummy exudation observed in mango orchards was suspected to be the important factor of mango decline in South, Middle and North Gujarat area and hence investigated.

Symptoms observed in nature ~~was~~^{were} leaf blight, tip withering, defoliated twigs accompanied by brownish discolouration, basal trunk developing bark canker and gummy exudation. The pathogen associated with the disease was identified as Botryodiplodia theobromae Pat. after studying the pathogenicity tests on seedling and fruits of mango, symptomatology and morphological characters.

Studies regarding the organism associated with tissues and pure culture indicated initial production of hyaline, one celled, thin-walled, globose to subglobose immature spores, which later turned to brownish black, two celled, thick-walled with longitudinal striations at maturity. Pycnidial bodies were subglobose to globose, erumpent, ostiolated measuring $145 - 396.4 \times 107 - 323.5 \mu\text{m}$ with an average of $289.74 \times 219.13 \mu\text{m}$ while the average size of pycnidiospores measuring $26.4 \times 11.3 \mu\text{m}$.

Physiological studies revealed the Richard's medium and Czapek's medium to be the best for the growth and sporulation and Asthana and Hawker's medium as the poorest.

In incubation study the maximum growth was found after 15 days of incubation in the liquid media. The optimum range of temperature requirement was between 25° - 30° C for both growth and sporulation. At 40° C and 10° C, the growth was meagre. Slightly acidic medium pH 6.0 to 6.5 was optimum for the growth and sporulation. However, the organism could grow in the wide range from pH 4 to 9. 'Glucose', 'Maltose' and 'Sucrose' were better and 'Lactose', 'Xylose', 'Galactose' and 'Starch' proved to be inferior sources of carbon. 'Calcium nitrate' was better and 'Ammonium sulphate' the poorest source of nitrogen. 'Calcium nitrate' and 'Maltose' combination of nitrogen and carbon source was better while the combination of 'Calcium nitrate' and 'Glucose' was poorest.

Among the fungicides tested (in vitro), carbendazim (250 ppm) was most effective in checking the growth of fungus. Next best were mancozeb (1000 ppm), PMA 1 % (1000 ppm) and MEMC 6 % (1000 ppm).

In field trial, soil drenching of carbendazim (0.05 %) was significantly superior to PMA 1 %, carbofuran, bordeaux mixture and combination of carbendazim + carbofuran, PMA + carbofuran and B.M. + carbofuran.

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* Original not seen.

APPENDICES

APPENDIX - I

1. Asthana and Hawker's medium :

Potassium dihydrogen phosphate(KH_2PO_4)	:	1.750	g
Potassium nitrate (KNO_3)	:	3.5	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.5	g
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	5.0	g
Distilled water	:	1000.0	ml

2. Brown's medium :

Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	20.0	g
Potassium dihydrogen phosphate (KH_2PO_4)	:	1.250	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.750	g
L(-) Asparagine ($\text{NH}_2 \cdot \text{CO} - \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$)	:	2.0	g
Distilled water	:	1000.0	ml

3. Czapek's medium :

Sodium nitrate (NaNO_3)	:	2.0	g
Potassium monohydrogen phosphate(K_2HPO_4)	:	1.0	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.5	g
Potassium chloride (KCl)	:	0.5	g
Ferrus sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.01	g
Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	:	30.0	g
Distilled water	:	1000.0	ml

4. Elliott's medium :

Potassium dihydrogen phosphate (KH_2PO_4)	:	1.350	g
Sodium carbonate (Na_2CO_3)	:	1.06	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.125	g
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	5.0	g
L(-)Asparagine($\text{NH}_2 \cdot \text{CO} - \text{NH}_2 - \text{CH}(\text{NH}_2) \cdot \text{COOH}$)	:	1.0	g
Distilled water	:	1000.0	ml

5. Modified Asthana and Hawker's medium :

Potassium dihydrogen phosphate (KH_2PO_4)	:	1.0	g
Sodium nitrate (NaNO_3)	:	3.0	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.5	g
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	5.0	g
Distilled water	:	1000.0	ml

6. Potato Dextrose Broth :

Peeled potato	:	200.0	g
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	20.0	g
Distilled water	:	1000.0	ml

7. Potato Sucrose Broth :

Peeled potato	:	200.0	g
Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	:	30.0	g
Distilled water	:	1000.0	ml

8. Richard's medium :

Potassium nitrate (KNO_3)	:	10.0	g
Potassium monobasic phosphate (K_2HPO_4)	:	5.0	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	2.50	g
Ferric chloride (FeCl_3)	:	0.02	g
Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	:	50.0	g
Distilled water	:	1000.0	ml

9. Standard nutrient medium :

Potassium monohydrogen phosphate (K_2HPO_4)	:	1.36	g
Sodium carbonate (Na_2CO_3)	:	1.06	g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	5.00	g
L(-) Asparagine ($\text{NH}_2 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$)	:	1.00	g
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$)	:	5.00	g
Distilled water	:	1000.00	ml

APPENDIX - II

Sr. No.	Chemical name	Trade name	Active ingredient
1.	Aureofungin	Aureofungin	N-methyl-P-amino-acetophenone + mycosamine
2.	B.M.	Bordeaux mixture	Copper sulphate + calcium hydroxide in solution
3.	Captafol	Difolatan	N(-1,1,2, 2-tetrachloroethyl) thiocis-4 cyclohexane-1, 2 dicarboximide 80 %
4.	Captan	Captan	Cis- N (Trichloromethyl thio) 4-cyclohexene-1, 2 dicarboximide
5.	Carbendazim	Bavistin	Methyl-1 H- Benzimidazole 2 yl - carbamate 50 WP
6.	Carboxin	Vitavax	5-6-dihydro-2 -methyl -1 -4 oxathiin -3 - carboxianilide
7.	Mancozeb	Dith-M-45	Zinc ion manganese ethylene-bisdithiocarbamate 75 %
8.	MEMC	MEMC 6 %	Methoxy ethyl mercury chloride
9.	PMA	PMA 1 %	Phenyl mercury acetate
10.	Thiophenate methyl	Topsin-M	1,2, bis (3-methoxy carbonyl-2-thioureido) 70
11.	Thiram (TMTD)	Danatax	Bis (dimethylthio-carbamyl) disulfide OR Tetramethyl thirumdisulfide
12.	Triforine	Saprol 15 EC	N, N-(1,4, piperazinediyl-bis (2,2,2 - trichloroethylidene) bis formamide