STUDIES ON DIE BACK DISEASE OF MANGO (Mangifera indica L.) CAUSED BY Botryodiplodia theobromae Pat.

By

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Reg. No. 92118

A Thesis Submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH

RAHURI, 413 722 DIST.- AHMEDNAGAR,

Maharashtra State (India)

in partial fulfilment of the requirements for the degree

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in

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DEPARTMENT OF PLANT PATHOLOGY & AGRICULTURAL MICROBIOLOGY

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RAHURI, DIST- AHMEDNAGAR, M. S. (INDIA)

1994

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CANDIDATE'S DECLARATION

I hereby declare that the thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

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This is to certify that the thesis entitled "Studies on die back disease of mango (Mangifera indica L.) caused by Botryodiplodia theobromae Pat." submitted to the Faculty of Agriculture, MAHATMA PHULE KRISHI VIDYAPEETH RAHURI, DIST. AHMEDNAGAR, MAHARASHTRA STATE (INDIA) in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of a bona fide research work carried out by SHRI S.A.SHELAR under my guidance and no part of the thesis has been submitted for any degree or diploma or publication.

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This is to certify that the thesis entitled "Studies on die back disease of mango (*Mangifera indica* L.) caused by *Botryodiplodia theobromae* Pat." submitted to the Faculty of Agriculture, MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI, DIST. AHMEDNAGAR, MAHARASHTRA STATE (INDIA), in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of a piece of *bona fide* research work carried out by Shri S. A. Shelar, under guidance of Dr.D.N.Padule, Plant Pathologist, Seed Borne Diseases (NSP), Seed Cell, Mahatma Phule Krishi Vidyapeeth, Rahuri, and that no part of the thesis has been submitted for any other degree or diploma.

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ABBREVIATIONS

B.C.	:	Before Christ
A.D.	:	anno Domini
Cm	:	Centimeter(s)
^{o}C	:	Degree celsius
C.D.	:	Critical difference
Cvs.	:	Cultivars
et al.	:	et alii
etc.	:	et cetera
Fig.	:	Figure
g	:	Gramme(s)
ha	:	Hectare
i.e.	:	That is
kg	:	Kilogramme(s)
Ltd.	:	Limited
m	:	Meter(s)
mg	:	Milligramme(s)
ml	:	Millilitre(s)
mm	:	Millimetre(s)
PDC	:	Per cent disease control
Lux	:	Light intensity
S.E.	:	Standard Error
u	:	Micron
Vit.	:	Vitamin
%	:	Per cent

ABSTRACT

STUDIES ON DIE BACK DISEASE OF MANGO (*Mangifera indica* L.) CAUSED BY *Botryodiplodia theobromae* Pat.

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The present investigations were carried out with a view to study the symptomatology of the disease, morphological, cultural and physiological characteristics of the pathogen i.e., *Botryodiplodia theobromae* Pat., responsible for the die back disease of mango, their chemical control *in vivo* and *in vitro* and screening of germplasm of mango against this disease under natural conditions of disease development.

The casual fungus was isolated from infected twigs of mango. Pathogenecity of die back pathogen was proved by artificial inoculation method. The fungal culture was identified as *Botryodiplodia theobromae* Pat.

Contd.....

Disease symptoms were characterised as wilting of twigs and branches followed by complete defoliation and gave the tree an appearance as if it was scorched by fire. Colouring and darkening of bark at a certain distance from the tip was the external evidence of the disease . Such dark patches were generally seen on young green twigs and were hardly distinguishable in older branches. The upper leaves lost their healthy green colour and gradually turned brown. This was followed by browning of the whole leaf accompanied by the upward rolling of the margin. The brown rolled leaves shrivelled. The shrivelled twig all together was the characteristics of an advance stage of the disease. Internal browning in the wood tissues was observed on splitting along the long axis.

In morphological studies, the fungal pathogen produced cottony white mycelium. The mycelium was irregularly branched, septate, primarily hyaline but with age turned olive grey to olive black in colour within seven days. Pycnidial bodies were formed in six days. Pycniospores were hyaline at early stages of growth and turned brownish. They were single celled primarily and became bicelled at maturity within nineteen days of inoculation. They were oval to

Contd....

oblong granular at early stages. The pycniospores measured 11.16-15.11 um in length and 7.39-11.75 um in breadth. In spore germination studies, it was revealed that good spore germination was observed at the end of ten hours in host leaf extract followed by 1% sugar solution.

Among the various culture media used, good growth and abundant sporulation were observed on Richard's agar and potato dextrose agar, while good growth and poor sporulation on Sabourd's media.

In the physiological studies the minimum, optimum and maximum temperatures recorded for good growth and sporulation were 15, 25 ± 2 and 35 °C, respectively.

The best carbon sources observed for growth and sporulation were sucrose and glucose followed by mannitol. Good sporulation was observed on maltose, lactose and mannitol whereas moderate sporulation was observed on glucose.

The best nitrogen sources observed were potassium nitrate followed by peptone and sodium nitrate. Good sporulation was observed on calcium nitrate and sodium nitrate whereas moderate sporulation was observed on peptone and ammonium nitrate.

Contd...

Fluorescent light (850 lux) was required for abundant septate sporulation and good growth followed by room light and sunlight.

In in-vivo evaluation of fungicides carbendazim (0.1%) was found to be the most effective in controlling the disease intensity of die back of mango after fourth spraying. It was followed by thiophanate-methyl (0.1%), captan (0.2%), benomyl (0.1%) and mancozeb (0.25%).

In in-vivo evaluation of fungicides carbendazim (0.1%), thiophanate-methyl (0.1%), captan (0.2%), benomyl (0.1%), mancozeb (0.25%) have checked the complete growth of the pathogen. The fungicides aureofungin (0.05%), and copper oxychloride (0.25%) appeared promising in checking the growth of pathogen.

In liquid media studies carbendazim (0.1%), thiophanate-methyl (0.1%), captan (0.2%), benomyl (0.1%) and mancozeb (0.25%) were found 100 per cent effective in checking the growth of the pathogen. The fungicides aureofungin (0.05%) was also found promising in production of less dry mycelial weight of the pathogen.

Contd....

In varietal screening programme one variety namely Bombay butto was observed to be resistant under natural conditions of disease development. Badami, Salem, Kesar, Amrapali, Benaras i, Langara proved to be moderately susceptible. Alphonso was highly susceptible to this disease. None of the varieties was observed highly susceptible to die back disease of mango.

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Introduction

1. INTRODUCTION

Mango (Mangifera indica L.) is said to have originated in the Indo Burma region (North East India and North Burma) (De candolle 1904, Popenoe 1920, Mukherjee 1951). Commercial cultivation of mango started only a few decades ago under the North and South Indian conditions. At present its cultivation has become one of the most remunerative farming enterprises and the area under mango is steadily increasing. India probably has more commercial plantings than the rest of world (Ochse et al., 1961). The world production of mango was 16.84 million tonnes during 1992 (FAO, 1993). India's contribution was 0.98 million tonnes. Mango occupied 1.06 million hectare area in India with total annual production of nine million tonnes (Madhava Rao, 1992). In India, mango is distributed throughout the length and breadth of the country except in hilly region above 915 metres from the mean sea level. Mango is grown under tropical as well as sub tropical climate in India. The leading mango growing states in India are Uttar Pradesh, Andhra Pradesh, Bihar, Orissa and West Bengal. During the last two decades, mango cultivation has gained a considerable momentum in the states of Haryana, Karnataka Maharashtra, Punjab, Rajasthan and there is much scope for extending the mango growing to some part of Gujarat, Madhya Pradesh. In Maharashtra, commercial mango cultivation was restricted to the Konkan area. During 1962-63, mango cultivation was extended to other districts of Maharashtra.

The total area under mango cultivation in Maharashtra was 49.873 ha with total production of 9,144 tonnes (Anonymous, 1993).

Mango has assumed a premier position among the cultivated fruits of the country. It is considered as national fruit of India and rightly styled as the king of fruit by virtue of its delicious taste. Mango and their products are exported to South-East Asia, Europe and the U.S.A. There are hundreds of varieties of mango. Out of which only a few happen to be commerically cultivated. The most well known varieties throughout the country are Alphonso, Kesar, Dashhari, Langra, Totapuri, Neelum, Samar Behisht, Chausa, Fazli, Krishnabhog, Himsagar, Pairi, Gulabkhas, Zardalu, Bangalora, Suvarnarekha, Raspuri and Badami. Among these Neelum and Bangalora happen to be the most consistent bearers (regular) and Dashhari by far the most delicious variety. The climate of Maharashtra is the most suitable for the mango cultivation. In Maharashtra State, the varieties like Alphonso (Hapus), Kesar, Vanraj, Langara, Payari, Totapuri, Neelum are commercially grown on large scale.

The major diseases on mango in the field are anthracnose caused by Colletotrichum gloeosporioides Penz., Powdery mildew caused by Oidium mangiferae Berth., die back disease caused by Botryodiplodia theobromae Pat., Sooty mould caused by Capnodium mangiferae cke., blight caused by Macrophoma mangiferae

Hingorani, pink disease caused by Botryobasidium salmonicolar (Berk and Br.), pestalotia leaf spot caused by Pseudomonas manqiferae indicae (Patel et al., mango malformation caused by Fusarium moniliformae and many others.

In Maharashtra State, the die back disease caused by Botryodiplodia theobromae is recently becoming one of the major diseases of mango which causes heavy losses in mango production. The disease is characterised by wilting of branches and twigs particularly of the older tree followed by complete defoliation and gives the tree an appearance as if it has been scorched by fire. Brown colouring and darkening of bark at a certain distance from the tip is the external evidence of the disease. Such dark patches are generally seen on young green twigs and hardly distinguishable in older branches. The upper leaves loose their healthy green colour and gradually turn brown. This is followed by browning of the whole leaf accompanied by the upward rolling of the margin. The brown rolled leaves become shrivelled. The shrivelled twig is the characteristic of the advance stage of the disease. Internal browning in the wood tissue is observed on splitting along the long axis, cracks appear on wooden branches from which gum exudes before they die out. When graft union of young plant is affected the plant usually dies. Shedding progresses rapidly until the whole twig dies. The effect of the disease on the general appearance of the tree

is noticed at any time of the year but it is most conspicuous during September and October. There are no precise data about the exact loss caused by die back disease. However, Pathak and Srivastava (1967) recorded 4-6 per cent losses due to this disease in India. Therefore, it was felt necessary to undertake the study on different aspects of die back disease of mango caused by Botryodiplodia theobromae with the following objectives.

1. To isolate the pathogen of die back disease of mango
2. To identify the pathogen and prove its pathogenecity.
3. To study the morphological, cultural and physiological characters of the fungus.
4. To evaluate the fungicides in-vivo.
5. To evaluate the fungicides in-vitro.
6. To screen the germplasm of mango under natural conditions of disease development against this disease.

Review of Literature

2. REVIEW OF LITERATURE

The literature on the occurrence of the disease, isolation of the pathogen and its pathogenecity, symptomatology, morphological, cultural and physiological characters of the fungal pathogen, fungicidal control in-vivo and in-vitro, varietal screening against the die back disease, under study is briefly summarised :

2.1. Occurrence

Botryodiplodia theobromae was first described from Cacao pods in Ecuador by Patouillard and de Lagerheim (1892) and since that time the fungus has been reported under various names on a wide range of host species retained at Commonwealth Mycological Institute (Punithalingam, 1980). The fungus commonly occurs in almost all the countries in the world.

Bourne (1921) reported the die back disease of mango (B. theobromae) from Borbados.

Das Gupta and Zacharich (1945) reported the die back disease of mango (B.theobromae) from the Department of Botany, Lucknow University.

Verma and Singh (1970) reported the die back disease of mango (B.theobromae) from Govt.Agricultural Research Farm, Durgapura, Jaipur.

Om Prakash and Raoof (1979) reported die back disease of mango (B.theobromae) from Central Mango Research Station, Lucknow.

2.2 Isolation and Pathogenecity

Isolation :

Lantican and Quimio (1976) isolated B.theobromae from post-harvest rots of mango.

Om Prakash and Raoof (1979) isolated B.theobromae causing die back disease of mango. Green living twigs and branches showing discoloured cankers and cracks were chosen for isolation. Trinidade and Gasparotto (1982) isolated B.theobromae from infected plant of rubber.

Pathogenecity :

Botero (1972) proved the pathogenecity of B.theobromae causing die back disease of Ceropia tessania or dry necrosis of woody tissues of Ceropia tessania was found in plant of all sizes. Plant infected near the apex died, especially if they were young.

Om Prakash and Raoof (1979) proved the pathogenecity of B.theobromae causing die back disease of mango. They found the fungus was more pathogenic on young shoots. Pathogenicity test was conducted on mango seedlings raised from seeds and grown in pots. Ten days old culture maintained on oat meal

agar were chosen for experimentation. The places to be incisioned were first cleaned by swabbing with O.1 per cent mercuric chloride and washed with the help of sterlized water. A small slit was made with the help of sterlized knife at a node adjacent to the bud and mycelium alongwith medium was inserted in the slit. The wounded site of inoculation was wrapped with moist cotton with polyethylene bag. After 48 hours of inoculation, the polyethylene bag was removed and observation was recorded carefully. Dark local lesions developed at the site of inoculation and extended along the branch. Drying of twig was observed within 15-20 days.

Schiffmann et al., (1985) proved pathogenicity of B.theobromae on mango fruit.

2.3 Symptomatology

Rath et al., (1978) described the symptoms of die back disease of mango caused by B.theobromae. The disease manifests three distinct symptoms viz., wither tip, twig blight and die back disease which they considered as separate diseases. Wither tip symptoms were restricted to the tender tip of one year growth of plant. The affected tip dry up, loose weight and ultimately drop off leaving distinct leaf scars on surface of diseased tips. In the twig blight phase, the affected twig showed different degree of blighting and wilting accompanied by brownish discolouration, shrivelling

and ultimate death. In the die back phase, large branches and the basal trunk developed bark cankers and gummy exudation. Eventually even large branches of the entire tree die.

Om Prakash and Raoof (1979) described the symptoms of die back disease caused by B.theobromae. The disease is characterised by wilting of branches and twig, particularly of older tree, followed by complete defoliation and gives the tree an appearance as if, it has been scorched by fire. Colouring and darkening of bark at a certain distance from the tip is the external evidence of the disease. Such dark patches are generally seen on young green twigs and hardly distinguishable in older branches. The bark is discoloured at several points and the dark lesion increases affecting the mid ribs. The upper leaves loose their healthy appearance and gradually turn brown. This is followed by browning of the whole leaf accompanied by the upward rolling of the margin. The brown rolled leaves shrivelled. The shrivelled twig is the characterstic of the advance stage of the disease. Internal browning in the wood tissue is observed on splitting along the long axis.

Burhan (1987) described the symptoms of mango die back disease caused by B.theobromae. In young mango seedling, blackening and necrosis of tap root was observed. On older plants, die back, twig blight and blackening of stem were observed followed by stem canker, necrosis and root necrosis.

2.4 Morphology and spore germination

2.4.1 Morphology

Wardlaw (1932) reported the morphology of B.theobromae from banana. The pycnidia were described as densely, gregarious, more or less immersed ostiolate, 200 to 300 um in diameter. Conidia were at first hyaline, uniseptate, brown in colour and non constricted. They measured 12-70 x 10-13 um.

Om Prakash and Raoof (1979) reported the morphology of B.theobromae from mango. Hypha of the pathogen was septate, granular, later gutulate hyaline at first, turning dark grey to black in colaur, pycnidia were short beaked, globose or subglobose, size varied from small to 300 um leathery or carbonaceous membranous. Pycniospores were hyaline when young, subgutulate, one celled, granular, measuring 15-75 x 9-15 um when they matured, two celled, dark, steriated not constricted and darker at the septum.

Sherkar (1979) reported morphology of B.theobromae from pomegranate. The fungus produced cottony white mycelium which was 2.30-4.37 um(average 3.13 um) in width, irregularly branched, septate, primarily hyaline but with age turned olive grey to olive black in colour. After 72 hours of incubation, dark brown to black pycnidial bodies were observed on the substratum. They were oval to spherical or elongated in shape. They measured 268.04 x 216.76 um (117.99 - 458.85 x 91.77-393.30 um in diameter. Pycnidiospores were hyaline,

smooth, oval to oblong granular at early stage and turned brownish one septate as they obtained maturity. They measured 9.88 - 25.09 x 6.08-12.92 um (19.35 x 10.79 um)in diameter.

Gadage (1984) described the morphology of B.theobromae from grape. The average size of the pycnidiospores was 20-30 x 10-15 um. Immature conidia were hyaline, unicellular, ellipsoid to oblong thick walled with granular contents on maturity. The conidia became two celled dark brown and were differentially pigmented.

2.4.2 Spore germination

Gadage (1984) showed that maximum germination of conidia i.e., 100 per cent of B.theobromae was observed in grape fruit extract. It was followed by grape leaf extract (95%), one per cent sugar solution (91%), tap water (81%), distilled water (79%) and sterile water (78%).

2.5 Cultural studies

2.5.1 In solid media

D'Souza (1963) reported that among the synthetic and non synthetic media tried for growth and sporulation of B.theobromae of Colocasia antiquarum, Richard's agar, Coon's agar and potato dextrose agar supported good growth.

Srivastava and Tandon (1969) reported that the B.theobromae fungus could thrive between 15 and 35°C temperature. It did not grow at 10 and 40°C. It was not killed at these temperature for 15 days. The optimum temperature for growth and pycnidial production was 25°C but conidial germination was maximum at 30°C.

Verma and Singh (1970) reported that good growth and sporulation of die back disease fungus i.e., B.theobromae of mango was at 27-30°C temperature.

Prasad and Sinha (1981) reported that fruit rot of mango caused by B.theobromae was nil at 10°C for 16 days, it increased gradually with increase in temperature and at 35°C temperature it was 100 per cent in 12 days.

2.6.2 Utilization of carbon source

2.6.2.1 In solid media

Srivastava and Tandon (1968) reported that good growth and sporulation of B.theobromae on glucose. The fungus utilized lactose with the formation of synthetic oligosaccharides in all carbon sources tried.

Gupta (1977) reported that mean per cent germination of spores of B.theobromae increased with increased percentage of sucrose ranging from 1 to 30 per cent.

Sherkar (1979) observed good growth and sporulation of B.theobromae on sucrose and glucose followed by maltose, mannitol, dextrin and lactose.

Gadage (1984) observed good growth and sporulation of B.theobromae on fructose followed by glucose, dextrose and sucrose.

2.6.2.2 In liquid media

Among the carbon sources tried, sucrose produced significantly higher dry mycelial weight and sporulation over control followed by glucose, cellulose, starch, xylose, lactose, galactose. The soluble cellulose and starch supported moderate growth and sporulation but maltose proved to be the poor source of carbon (Shinde, 1988).

2.6.3 Utilization of nitrogen sources

2.6.3.1 In solid media

Patil (1966) showed good growth and sporulation of B.theobromae on peptone and tryptophan. The inorganic nitrogen compounds calcium nitrate, ammonium oxalate, ammonium tartarate, potassium nitrate and sodium nitrate supported good growth and sporulation. Sodium nitrite, ammonium nitrate and urea did not support good growth.

Srivastava and Tandon (1968) reported that good growth and production of carboxymethyl cellulose in the culture of B.theobromae was better with $NaNO_3$ as nitrogen source than with NH_4Cl or NH_4NO_3.

Sherkar (1979) observed that good growth and sporulation of B.theobromae on potassium nitrate and sodium nitrate.

2.6.3.2 In liquid media

Among the nitrogen sources tried, potassium nitrate produced significantly higher dry mycelial weight and sporulation over control followed by ammonium sulphate, sodium nitrate, ammonium nitrate, urea, calcium nitrate, ammonium chloride and peptone. Sodium nitrate and ammonium nitrate produced moderate growth and sporulation but peptone proved to be the poor source of nitrogen (Shinde, 1988).

2.6.4 Effect of light on the growth of Botryodiplodia theobromae

Ekundayo and Haskins (1969) reported that B.theobromae produced abundant pycnidia on several media, under continuous fluorescent light but not in darkness. Pycnidial production was stimulated by light of 15 foot candle for 4 days for appreciable sporulation.

Alsodura (1970) reported fruit bodies of B.theobromae were induced in malt or oat meal agar cultures with continuous light (day light, fluorescent tubes 850 lux) either immediately after an initial incubation for 72 hours and then in the dark at 25°C.

2.6.5 Occurrence of pycnidia of B.theobromae under natural and under artificial conditions

Gadage (1984) showed that the pycnidia of B.theobromae appeared on leaves, petioles and trunk on one year old grape vine under natural condition. On the vines of 12 and 16 years old it occured on leaves, petioles, cane and arms only but it was not observed on trunk under natural conditions. Pycnidial bodies were observed on canes of Thomson seedless grape under artificial conditions.

2.7 Chemical control in-vivo

Sweets et al. (1962) reported that out of different fungicides benomyl was most effective against B.theobromae on rose. Benomyl applied at weekly interval reduced canker development in rose by B.theobromae.

Ranney (1971) reported that out of different fungicides used against boll rot of cotton caused by B.theobromae, agrosan, benomyl and panogen (methyl mercury dicynadimide) have been claimed to give significant level of control.

Pordespio and Berrod. (1976) reported that pre-bloom and post-bloom sprays with mancozeb + karathane + thiodan controlled incidence of B.theobromae in mango and improved the fruit setting.

Sivaprakasam et al. (1976) reported that out of different fungicides used against set rot of tapioca caused by Diplodia natalensis, bavistin was found most effective.

Sherkar (1979) showed that out of eight fungicides used against B.theobromae on pomegranate fruit in field, benlate was found to be the most superior in reducing percentage disease index (PDI) and increasing the percent disease control. Dithane z-78, bavistin and dithane M-45 were next best fungicides.

Rawal and Ullasa (1984) reported that out of different fungicides used against B.theobromae bavistin 0.1 per cent and Jkstein 0.1 per cent were found most effective.

Gadage (1984) used 11 fungicides against B.theobromae, the cause of fruit rot of grape berries. All the fungicidal treatments were found significantly superior to control. However, the most effective fungicides were aureofungin, bavistin, benlate, mancozeb, copper-oxychloride and ziride.

2.8 Chemical control in-vitro

2.8.1 In solid media

Chin (1967) reported that the fungicide dithane M-45 was completely inhibitory to the growth and sporulation of B.theobromae fungus the cause of banana fruit rot.

Ekundayo (1970) reported that the fungicide dinocap, captan and copper sulphate were completely inhibitory to the germ tube development of B.theobromae.

Srivastava and Tandon (1971) reported that the fungicide captan was completely inhibitory to the growth and sporulation of B.theobromae the cause of fruit rot of guava.

More (1976) showed that the fungicides, difolation, benlate, duter and captan were effective in inhibiting both growth and sporulation of B.theobromae the cause of grape berries rot.

Sherkar (1979) showed that benlate completely inhibited the growth of B.theobromae the cause of fruit rot of pomegranate. However, bavistin and dithane M-45 showed the inhibition 58.38 and 62.55 per cent, respectively.

Ogundana (1982) reported that out of twelve fungicides tested against B.theobromae on yam, benomyl and thiobendazole completely prevented infection either by mycelium growth or by sporulation. Captan, iodine and mancozeb prevented infection by sporulation but not by mycelium growth.

Agarwal et al. (1982) reported that aureofungin at 100 ppm gave maximum inhibition of the B.theobromae in in-vitro followed by potassium metabisulphide and sodium benzoate at 2000 ppm.

Gadage (1984) showed that out of 11 fungicides, aureofungin, bavistin and benlate completely inhibited growth

and sporulation of the fungus B.theobromae, the cause of fruit rot of grape berries. The fungicides alliete and delan were ineffective in restricting the growth and sporulation of the pathogen. The other effective fungicides in restricting the growth and sporulation of the fungus were zineb, mancozeb, copper oxychloride, ziride, RH 6161.

2.8.2 In liquid media

Shinde (1988) showed that mancozeb and carbendazim completely inhibited the growth of fungus B.theobromae the cause of die back disease of rose on liquid media. Dry mycelial weight production in captan and zineb were at par with each other. Copper oxychloride was not effective as compared to the other fungicides.

2.9 Varietal screening

Verma and Singh (1970) reported that die back disease caused by B.theobromae Pat. is the most serious disease of mango (Mangifera indica L.) in the Jaipur district of Rajasthan. Besides the pathogen, the environmental conditions the host itself are an important factor determining the severity of the disease. Mohan Bhog was the most susceptible and Saroli the least susceptible varieties of mango. Though no variety was completely resistant to the die back pathogen the differences in their susceptibility indicated that some varieties have certain resistant factor.

Verma and Singh (1973) reported that B. theobromae has been reported to cause both die back and fruit rot of mango under different conditions. Six varieties of mango namely Desi, Mohan Bhog, Langra, Saroli, Bari, Bomboi and Dashhari were taken for studying their response against the pathogen. Under natural conditions Mohan Bhog variety was observed to have maximum infection while Saroli had the minimum infection.

Material and Methods

3. MATERIAL AND METHODS

The material and methods followed during the present investigations are given below :

3.1 Material

The following material was used during the present investigations.

3.1.1 Disease samples

Diseased samples of mango were collected from the Instructional-cum-Research orchard of mango of the Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri for isolation of the pathogen.

3.1.2 Culture media

Common laboratory medium that is potato dextrose agar (PDA) was used for isolation of the pathogen responsible for the die-back disease of mango. Richard's medium was used for poison food technique studies, other media used were Czapek's agar, Coon's medium, Kirchoff's medium, Sabourad's agar, Nutrient agar and Oat meal agar.

3.1.3 Chemicals

The chemicals used for preparation of media and in carbon source studies were of analytical grade (AR) obtained from standard firms like M/S E.Merck (India) Pvt.Ltd., Worli, Bombay and M/s. Glaxo Laboratories (India) Pvt.Ltd., Bombay.

3.1.4 Fungicides

The following fungicides were used for the evaluation against the die back of mango in-vivo and in-vitro.

1. Aureofungin

a. Chemical name	:	Aureofungin
b. Active ingredient	:	16.15% SP
c. Concentration	:	0.05%
d. Manufacturer	:	Hindusthan Antibiotics Ltd., Pimpri, Pune

2. Captan (Captaf)

a. Chemical name	:	N-(trichloro-methylthio)-4-cyclothexane- 1,2 dicarboximide
b. Active ingredient	:	75% wettable powder
c. Concentration	:	0.2%
d. Manufacturer	:	Rallis India Ltd.,Bombay

3. Copper oxychloride (Blitox-50)

a. Chemical name	:	Copper oxychloride
b. Active ingredient	:	50% wettable powder
c. Concentration	:	0.25%
d. Manufacturer	:	Sandoz, India Ltd.Bombay

4. Carbendazim(Bavistin)

a. Chemical name	:	2-(Methoxy-Carbamoyl)-benzimidazole
b. Active ingredient	:	50% wettable powder
c. Concentration	:	0.1%
d. Manufacturer	:	BASF India Ltd.,Bombay.

5. Mancozeb(Indofil M-45) :

a. Chemical name	:	Zinc ion manganese ethylene dithiocarbamate
b. Active ingredient	:	75% wettable powder
c. Concentration	:	0.25%
d. Manufacturer	:	Indofil Chemical Ltd. Bombay

6. Thiophanate - methyl (Topsin M-70)

a. Chemical name	:	1,2-bis (3-metho-oxy carbonyl -2 thioureido) benzene
b. Active ingredient	:	70% wettable powder
c. Concentration	:	0.1%
d. Manufacturer	:	Motilal Pesticides (India) Pvt.Ltd., New Delhi.

7. Benomyl

a. Chemical name	:	Methyl-N-Cl-butylcarbamoyl)-2-benzimidazole carbamate
b. Active ingredient	:	50% wettable powder
c. Concntration	:	0.1%
d. Manufacturer	:	Bharat Pulverising Mills Ltd., Bombay

3.1.5 Glasswares

Different types of corning brand glasswares were used in experimental work. The common glasswares were petriplates, test tubes, conical flasks, measuring cylinders, glass rods, slides and coverslips etc.

3.1.6 Equipment

The laboratory equipment used were autoclave, oven, inoculation chamber, laminar air flow, refrigerator, research microscope, chemical balance, hand atomiser (Baby spray pump).

3.1.7 Miscellaneous material

Inoculation needle, forceps, blotter paper, spirit lamp, mercuric chloride ($HgCl_2$), plastic bags, plastic buckets, earthen pots, labels etc., were used during the course of the present investigation.

3.2 Methods

3.2.1 Isolation

Several isolations were made from the samples of die back disease of mango branches collected from mango orchard of the Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri by tissue isolation method. The infected stems of mango were washed with tap water so as to remove dirt. The affected portion along with little healthy portion was cut into small pieces and disinfected by immersing in mercuric chloride (1:1000) for $1\frac{1}{2}$ minutes. Then these pieces were washed in three changes of sterile water in order to remove traces of disinfectant. These pieces were then plated in petriplates, poured with potato dextrose agar previously sterilized at 1.05 kg/cm^2 pressure

for 15 minutes, under aseptic condition. These plates were then inverted and incubated at room temperature (27 ± 1°C). They were under observation for the growth of microorganism, when the growth and sporulation of microorganism was noticed (after seven days of inoculation), it was transferred into slants to obtain pure culture.

3.2.2 Maintenance of pure culture

The isolate obtained was then subcultured on PDA slants and kept at room temperature (27 ± 1°C) for eight days for growth. Such slants were preserved in a refrigerator at 5 to 10°C and periodically subcultured once in a month to maintain the viability of the fungus.

3.2.3 Pathogenecity

The pathogenecity of this fungus was tested under glass house condition on Alphonso mango variety. The stem was inoculated with fresh sporulating culture of fungus. Eight days old culture was used for inoculating the mango stem. The pathogenecity of fungus was tested on injured and uninjured stem. Before inoculation of the fungal culture, the stem of mango seedling was injured with pin and then inoculated with spore suspension of the pathogen. The stem without injury was also inoculated with spore suspension of the pathogen.

3.2.4 Reisolation

Reisolation was carried out from the artificially inoculated stem (twig). The fungus thus obtained was

transferred and maintained separately on potato dextrose agar slants for comparison with original culture.

3.2.5 Identification of culture

Pathogenic culture which was isolated from artifically inoculated stem was identified and confirmed from Dr.V.G.Rao, Mycologist, Agarkar Research Institute, Pune- 411004.

3.3 Morphological studies and spore germination

3.3.1 Morphological studies

Morphological characters of culture grown on potato dextrose agar were studied. Spores from culture were mounted in lactophenol cotton blue on a clean glass slide. Spores were then mixed thoroughly in lactophenol to get uniform spread. Conidial measurements were recorded with the help of ocular and stage micrometer. The morphological character namely size, shape and septation of conidia were recorded from seven days old culture grown on potato dextrose agar medium and the average size of conidia was worked out. Similarly the septation and colour of the mycelium was also recorded.

3.3.2 Spore germination

Suspension of oval spore was prepared in tap water, sterile water, distilled water, host leaf extract and sugar solution and a drop of spore suspension was placed on clean glass slides from each suspensions. Later the slides were

placed in moist chamber kept at 27 ± 1°C and germinating spore were observed under microscope after eight and ten hours.

3.4 Cultural studies

3.4.1 Growth characters on solid media

The cultural characters of the fungus were studied by using the following solid media i.e., 1) Czapek's agar 2) Coon's medium 3) Sabourd's agar 4) Richard's agar 5) Potato Dextrose Agar (PDA) 6) Kirchoff's media 7) Nutrient agar and 8) Oat meal agar

These media were prepared according to the laboratory procedure described by Tuite (1969). Twenty ml each, of the above sterilized liquid medium was poured into 100 mm diameter petriplates. These plates were inoculated at centre with the fungal disc of 5 mm diameter and incubated at 27 ± 1°C for seven days. Seven days old culture plates were observed by averaging the linear growth of colony in two directions for each plate. Cultural characteristics namely topography, type of margin, colour of the colony, sporulation and other characters were recorded.

3.4.2 Growth studies in liquid media

The component and preparation of different liquid media used were the same as that of above solid media except that agar was not used. One hundred ml aliquot of

each medium was dispensed in 250 ml Erlenmeyer conical flasks and replicated thrice. These flasks were plugged with absorbent cotton and sterilized in an autoclave at 1.05 kg/cm^2 pressure for 15 minutes. These flasks were inoculated with the fungal disc of 5 mm diameter and were incubated at 27 $\pm$ 1°C for seven days. Seven days old culture was used for inoculation. The mycelial mat was harvested on previously weighed Whatman filter paper No.40. The filter paper alongwith mycelial mats were dried at room temperature till the constant weights were recorded. Twelve days were required to get constant weight at room temperature. The results were analysed statistically.

3.5 Physiological studies

3.5.1 Effect of temperature on growth and sporulation

Richard's agar plates in duplicate were inoculated at the centre with fungal disc of 5 mm diameter and incubated at 0°, 5°, 10°, 15°, 20°, 25° $\pm$ (room temperature) 30°, 35° and 40° celcius for seven days. The observations on colony diameter and sporulation were recorded after seven days of inoculation.

3.5.2 Carbon utilization

The ability of the fungus to utilize different carbon compounds was judged by replacing sucrose of the Richard's agar medium with different carbon sources. The amount of carbon sources added were equivalent to carbon present in sucrose content of the Richard's medium and determined on

the basis of molecular weight. The carbon compounds used in the present studies were as follows :

1. Glucose	2. Sucrose
3. Maltose	4. Lactose
5. Mannitol	6. Suitable control was kept

3.5.2.1 Solid media studies

Richard's medium with different carbon compounds was prepared sterilized and triplicated plates for each of the carbon compound were poured. The plates were inoculated with culture disc of 5 mm diameter and incubated at 27 $\pm$ 1^{o}C for seven days. Seven days old fungal culture was used for inoculation. Richard's medium without sucrose served as control. The observations on colony diameter, growth characters and sporulation were recorded after seven days of inoculation.

3.5.2.2 Liquid media studies

In liquid media studies, the Richard's medium with different carbon compounds was prepared by adding 100 ml of aliquot of each carbon compounds. These media were dispersed in 250 ml. Erlenmeyer conical flasks and replicated thrice. These flasks were plugged with absorbent cotton and sterilized in an autoclave at 1.05 kg/cm^{2} for 15 minutes. These flasks were inoculated with fungal disc of 5 mm diameter and were incubated at 27 $\pm$ 1^{o}C for seven days. Seven

$1^{o}C$ for seven days. Seven days old fungal culture was used for inoculation. Richard's medium without nitrogen source served as control. The observation on colony diameter, and sporulation were recorded after seven days of inoculation.

3.5.3.2 Liquid media studies

In liquid media studies, Richard's medium with different nitrogen compounds was prepared by adding 100 ml aliquot of each nitrogen compounds. These media were dispersed in 250 ml Erlenmeyer conical flasks and replicated thrice. These flasks were plugged with absorbent cotton and sterlized in an autoclave at 1.05 kg/cm^2 for 15 minutes. The flasks were inoculated with fungal disc of 5 mm diameter and were incubated at 27 $\pm$ $1^{o}C$ for seven days. Seven days old culture was used for inoculation. The mycelial mat was harvested on previously weighed Whatman filter paper No.40. The filter papers alongwith mycelial mat were dried at room temperature till constant weight was observed. Twelve days were required to get constant weight at room temperature. The data were analysed statistically.

3.5.4 Growth relationship of the fungus with light

The fungus was fouhd sporulating in sunlight on the canes under natural conditions. Under laboratory conditions, it was observed that it was not septate sporulating. The cultures were therefore exposed to light and was found to be sporulating. A study was therefore undertaken to know the

effect of light on growth and sporulation of septate sporulation of the fungus. The plates poured with equal quantity of potato dextrose agar in the usual manner were inoculated with 5 mm diameter of mycelial bit of seven days old culture of the fungus. The plates were incubated at room temperature for 24 hours and were exposed to different illuminations of light as follows :

Sr. No.	Type of light	Illumination
1	Sunlight	3000 lux
2	Fluoroscent light	850 lux
3	Room light	750 lux
4	Incubator	30 lux
5	Complete darkness	-

Observations for colony diameter and sporulation were recorded after seven days of inoculation.

3.5.5 Occurrence of pycnidia of B.theobromae under natural conditions and under artificial conditions

A study was undertaken to know the presence of the fungus B.theobromae in twigs. The infected twigs were surface sterilized with 1:1000 mercuric chloride and rinsed with sterilized water for three times and were incubated in a specially designed sterilized moist chamber.

The moist chambers used for twigs were made out of test tubes of 25 mm diameter and 150 mm length. A cotton wool was inserted in the tube. It was made wet with sterile water. The tubes were plugged in usual manner and sterilized at 1.05 kg/cm^2 for 15 minutes in an autoclave. Surface sterilized pieces of twigs were inserted in these tubes under aspetic condition and were incubated for seven days under sunlight, fluoroscent light and room temperature. These tubes were observed for the presence of the fungus after seven days of inoculation.

3.6 Evaluation of fungicides in-vivo (Under glass-house conditions)

3.6.1 Experimental site

The experiment was conducted under glass house condition at the Department of Plant Pathology and Agril. Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.6.2 Experimental details

1. Design - Completely Randomised Design
2. No.of replications - Three
3. No.of treatments - Eight
4. Variety - Alphonso
5. Details of spraying -

Methodology :

One seedling of Alphonso mango was designated for each treatment per replication. The stems of all the seedlings

under experiments were inoculated with eight days old fresh sporulating oulture of fungus. Stem inoculation was done by pin prick method that is by injury method because this fungus was wound parasite. Immediately after inoculation moist cotton was wraped on the wounded portion to create humidity. After 8 to 10 days of inoculation, slight blightning symptom was observed on the stem. Immediately after the initiation of the disease symptom first spraying of fungicides was given. Subsequently four sprayings were given at an interval of 10 days. The dates of sprayings were as follows :

1. First spraying : 4.2.1994
2. Second spraying : 14.2.1994
3. Third spraying : 24.2.1994
4. Fourth spraying : 6.3.1994

3.6.3 Method of observations

The disease intensity was recorded at the end of each spraying as well as on 10th day after the last spraying. However, the disease intensity i.e., initial observation was also recorded before first spraying. On each of the seedling, length of blighted portion of the stem in mm was recorded.

The dates of observations were :

1. First observation : 3.2.1994
2. Second observation : 13.2.1994
3. Third observation : 23.2.1994
4. Fourth observation : 6.3.1994
5. Fifth observation : 16.3.1994

The per cent infection control was calculated by the formula as given below :

$$\text{Per cent infection control} = \frac{\text{Length of infected area in control} - \text{Length of infected area in treatment}}{\text{Length of infected area in control}} \times 100$$

3.7.6 Statistical analysis

Length of infected area was subjected to the statistical analysis which was carried out by standard statistical method, i.e., for analysis of variance. The standard error (S.E.) of infected area was calculated. To compare two treatment means, critical difference (C.D.) at 5% level of significance was worked out.

3.7 Evaluation of fungicides in-vitro

3.7.1 Solid media studies

Poison food technique was followed to evaluate the fungicides against pathogen (B.theobromae) responsible for the die back disease of mango. Richard's medium was prepared and

distributed in 100 ml aliquot in 250 ml Erlenmeyer conical flask and sterlized at 1.05 kg/cm^2 for 15 minutes. After sufficient cooling (i.e. 40^{o}C) to each of the flasks, measured quantity of different fungicides of the recommended concentrations were added. Flasks were shaken thoroughly and then poured in triplicate plates. These plates were inoculated with 5 mm fungal disc of seven days old culture grown on potato dextrose agar and incubated at 27 $\pm$ 1^{o}C for seven days. Plates with Richard's medium without fungicides served as control. Observation on colony diameter and sporulation were recorded on 8th day after inoculation. Radial growth was measured and results were expressed as per cent inhibition of mycelial growth over control by using the following formula (Padule and Shinde 1986).

$$I = \frac{100\ (\ C - T\ )}{C}$$

Where,

I = Per cent inhibition of fungal growth

C = Growth in mm on 8th day after inoculation in control

T = Growth in mm on 8th day after inoculation in treatment

The results were analysed statistically.

3.6.2 Liquid Media Studies

In liquid media studies one hundred ml of Richard's medium was dispensed in 250 ml Erlenmeyer conical flask. The flasks were plugged with absorbent cotton and sterilized in an autoclave at 1.05 kg/cm^2 for 15 minutes. After cooling measured quantity of different fungicides of the recommended concentrations were added and shaken thoroughly. Then these flasks were inoculated with 5 mm fungal disc of seven days old culture grown on potato dextrose agar and incubated at 27 $\pm$ 1^{o}C. The mycelial mat was harvested on previsouly weighed Whatman filter paper No.40. The filter papers along with mycelial mat were dried at room temperature till constant weight was observed. Twelve days were required to get constant weight at room temperature. The results were analysed statistically.

3.8 Varietal screening

Varietal screening programme against die back disease of mango was undertaken under natural conditions of disease development. Seventy six mango varieties already planted in the mango orchard of the Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri were screened under natural condition.

3.8.1 Experimental details

1. Experimental site

The Instructional-cum-Research Orchard, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri.

2. Mango varieties

1. Bombay yellow
2. Bombay deshera
3. Bombay rizode
4. Salem
5. Golden selection
6. Safed lucknow
7. Swamini
8. Sardar
9. Safeda
10. Badami model
11. Villai columban
12. Ben choda
13. Dil pasand
14. Jahangir
15. Himsagar
16. Vasi badam
17. Malgoa
18. Amelpur
19. Bensai
20. Misteri
21. Malda
22. Cowasji Patel
23. Rajmane
24. Amini
25. Belkhas
26. Kelakende
27. Paiposha
28. Chittoor
29. Krishna bhoja
30. Kervinda
31. Bombay butto
32. Nortio
33. Fazli Kalam
34. Fazli long
35. Ambe huide
36. Seedling
37. Surekha
38. Peter Pasand
39. Chittoormalgova
40. Puthi
41. Sheshmani
42. Ambe lawi
43. Totapuri
44. Raspuri
45. Salem badam
46. Sinduri
47. Kesar
48. Samar basti alibag
49. Dashhari
50. Neelum
51. Aminkhan
52. Vanraj

53. Langra
54. Borsha
55. Goa mankur
56. Rajapuri
57. Alphonso
58. Pairi
59. Dadumiya
60. Dud Pedha
61. Fernandin
62. Banarasi langra
63. Vishwanath Mukhe
64. Hybrid 7/1
65. Hybrid 3/7
66. Hybrid 2/4
67. Chinasuvernarekha
68. Kitta beni
69. Nelgoa
70. Banet alphonso
71. Allseason mango
72. Amrapali
73. Mallika
74. Sunderaj
75. Pairi scented
76. Badami

3.8.2 Methodology

Varietal block of mango was kept free from any fungicidal spray. These varieties were screened under natural conditions of disease development. The observations on disease incidence of each of the mango varieties were recorded as follows. For each of the varieties number of dried branches due to disease were recorded for one square meter area from all four sides of the tree. For the observation of branches from one square metre area of the plant, of all the four sides of tree were randomly selected and total number of healthy branches and diseased branches were counted. The per cent disease incidence was calculated by the following formula :

$$\text{Per cent disease incidence} = \frac{\text{Total no.of diseased branches}}{\text{Total no. of healthy branches}} \times 100$$

Only one observation was recorded on 24.9.1993 before flowering. The per cent disease incidence of all the varieties was worked out. The varieties were classified into different grades on the basis of percentage disease incidence as given below.

Disease reaction	Percentage disease incidence
1. Resistant	1-5
2. Moderately susceptible	6-10
3. Susceptible	21-50
4. Highly susceptible	51-100

Experimental Results

4. EXPERIMENTAL RESULTS

The present investigations were carried out on die back disease of mango caused by Botryodiplodia theobromae Pat., in respect of symptomatology, isolation of the pathogen, pathogenecity, reisolation and identification of the pathogen, morphological characteristics and spore germination, cultural characteristics, physiological characteristics, chemical control in-vivo and in-vitro and screening of germplasm against this disease under natural conditions of disease development. The results of these aspects are presented hereunder :

4.1 Symptomatology

The symptoms of the disease were observed under natural conditions of disease development and under after artificial inoculation of the pathogen. Under natural conditions of disease development, the disease is characterised by wilting of branches and twigs particularly of the older tree followed by complete defoliation and gives the tree an appearance as if, it is scorched by the fire. Colouring and darkening of bark at a certain distance from the tip is the external evidence of the disease. Such dark patches are generally seen on young green twigs and are hardly distinguishable in older branches. The upper leaves loose their healthy green colour and gradually turn brown. This is followed by browning of

Plate 1. Symptom produced on tree by Botryodiplodia theobromae Pat.

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Plate 2. Symptom produced on tree by Botryodiplodia theobromae Pat.

Plate 3. Symptom produced on twig by Botryodiplodia theobromae Pat. showing internal browning in the wood tissue.

whole leaf accompanied by the upward rolling of the margin. The brown rolled leaves get shrivelled. The shrivelling of twig is the characteristics of the advance stage of the disease. Internal browning in the wood tissue is observed on slitting along the long axis. Crack is appear on wooden branches from which gum exudes before they die out. When graft union of young plant is affected the plant usually dies. Shedding progress rapidly until whole twig die. The effect of the disease on the general appearance of the tree is noticeable at any time of the year but it is most conspicuous during September and October (Plate 1, 2, 3).

4.2 Isolation, pathogenecity, reisolation and identification of cultures

4.2.1 Isolation

Isolation was made from the infected stem of mango yielded the culture of Botryodiplodia theobromae Pat. The fungus growth emerged from the cut ends of infected pieces within three to four days.

4.2.2 Pathogenecity

Young seedling of Alphonso mango inoculated after injury showed small dark brown to black patches on stem near the points where inoculation was made. Patches were produced 12 days after inoculation (Plate 4). However, the seedlings inoculated without injury did not show symptoms (Plate 5). Similarly, the control plants also did not show any disease symptoms.

1. Treatment

2. Control

Plate 4. Pathogenecity test of Botryodiplodia theobromae Pat. on mango seedling by injury method.

1. Treatment

2. Control

Plate 5. Pathogenecity test of Botryodiplodia theobromae Pat. on mango seedling without injury.

4.2.3 Reisolation

Reisolation was made from the inoculated mango seedling stem which yielded the same pathogen to that of original one.

4.2.4 Identification of culture

The pathogenic culture which was isolated from diseased stem (twig) was identified as Botryodiplodia theobromae Pat. and has got confirmed by Dr.V.G.Rao, Mycologist, Agarkar Institute, Pune - 411004.

4.3 Morphology and spore germination

4.3.1 Morphology

The fungus produced cottony white mycelium, which was irregularly branched, septate, primarily hyaline but with age turned olive grey to olive black in colour. Single celled pycniospores were hyaline, smooth, oval to oblong granular in early stages. They turned in brown to dark brown in colour as age advanced and measured 8.66-10.01 um in length x 5.5-9.19 um in breadth (Plate 6). These spores turned into bicelled pycniospores when they attain maturity. Generally the bicelled spores were produced within 19-20 days in the culture. They were dark brown in colour and oval to oblong in size, and measured 11.16-15.11 um in length x 7.39-11.75 um in breadth (Plate 7).

4.3.2 Spore germination

The spore germination study of single celled pycniospores was undertaken in five different media. The observations on

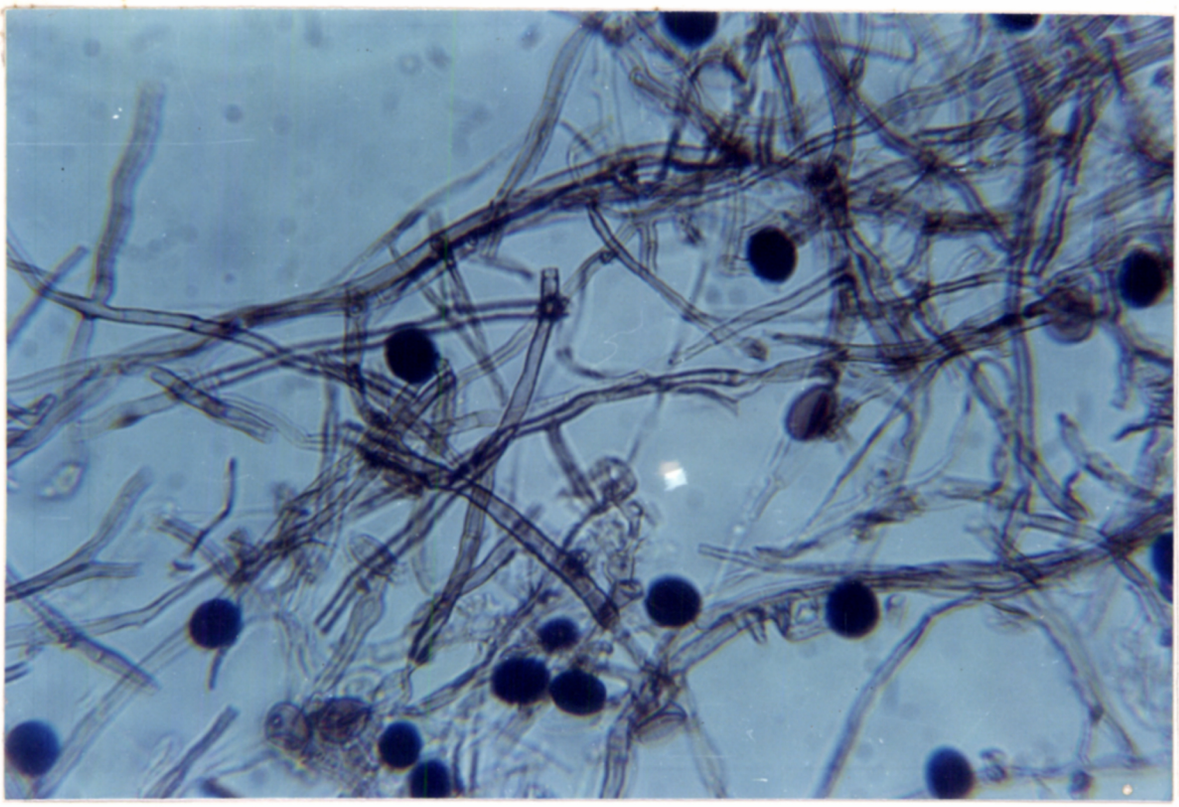


Plate 6. Microphotograph of mycelium and single cell pycniospores of Botryodiplodia theobromae Pat.

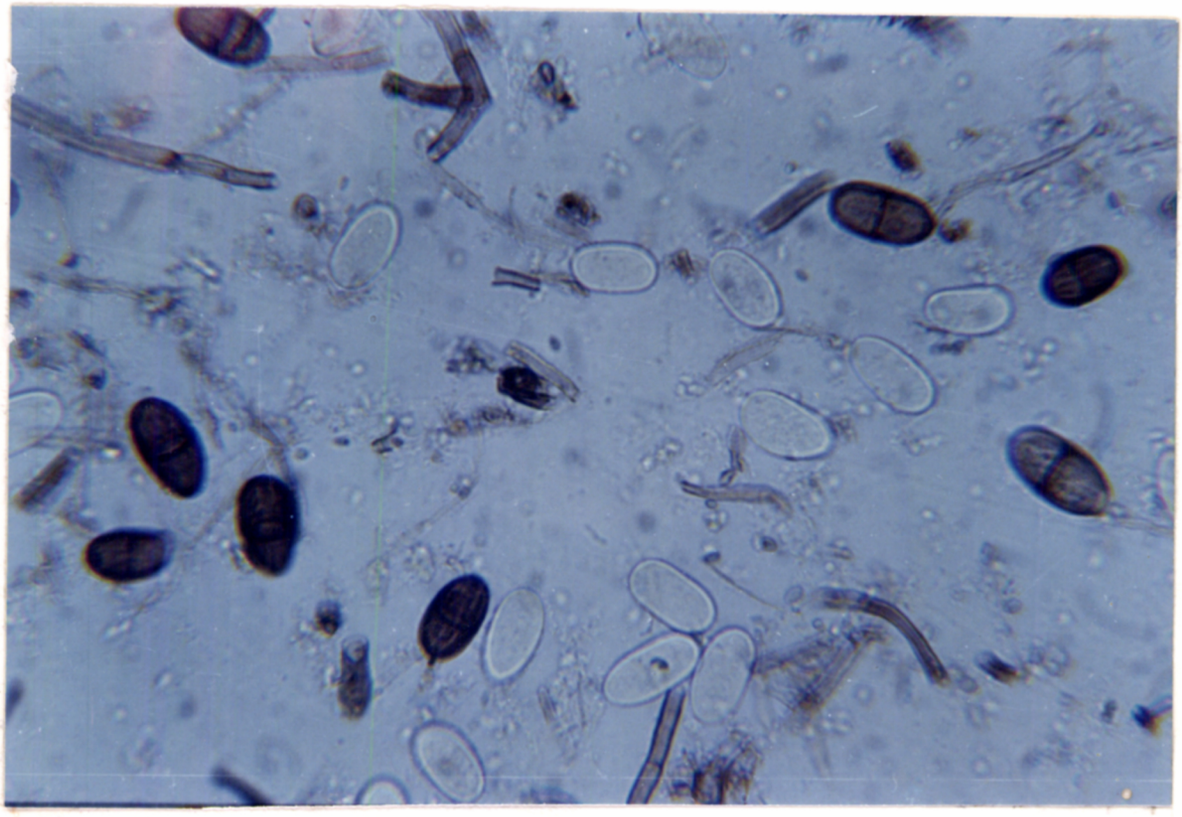


Plate 7. Microphotograph of bicell conidia of Botryodiplodia theobromae Pat.

of growth rate ranging from 37.25 to 90 mm in diameter. Growth characters on different solid media are illustrated in Plate 8.

The results presented in Table 2 and illustrated in Plate 8 revealed that fungus could grow on all the media tried. Among the solid culture media tried for characteristion of the pathogen. Richard's agar and Sabourd's media produced significantly good growth to rest of media tried and were on par in this respect. It was followed by potato dextrose agar. Among the culture media tried Richard's agar and Sabourd's media produced maximum colony diameter i.e. 90 mm. It was followed by potato dextrose agar (79.17 mm). However, Coon's agar produced least diameter of the colony of the pathogen that is 37.25 mm.

Abundant sporulation was observed on Richard's agar and potato dextrose agar. Good sporulation was recorded on Czapeak's agar, and Coon's agar. Poor sporulation was observed on Kirchoff's agar, Nutrient agar, oat meal agar and Sabourd's medium.

4.4.2 Growth characters on liquid media

The media which were used for growth characters in solid media were also used in liquid state for growth characterisation. Dry mycelial weight were recorded after seven days of inoculation. The results are presented in Table 3 and illustrated graphically in Fig.1.

Table 2. Cultural characters of Botryodiplodia theobromae Pat. on different solid culture media

Sr. No.	Media	*Mean colony diameter (mm)	Sporulation	Growth characters
1.	Czapek's agar	61.67	+++	Colony circular with entire margin flat and moderate growth, whitish in colour with concentric ring form in the centre.
2.	Coon's medium	37.25	+++	Colony irregular with entire margin, flat and moderate growth, whitish in colour.
3.	Sabourd's medium	90.00	+	Colony circular with entire margin flat and profuse growth, white in colour.
4.	Richard's agar	90.00	++++	Colony circular with entire margin raised and excellent growth, grey to whitish in colour.
5.	Potato dextrose agar	79.17	++++	Colony circular with break margin, dome shaped with raised centre and moderate growth, white in colour
6.	Kirchoff's agar	64.67	+	Colony irregular submerged and moderate growth, dirty white in colour
7.	Nutrient agar	45.17	+	Colony irregular, flat and loose growth, white in colour.
8.	Oat meal agar	52.17	+	Colony circular with entire margin, submerged and poor growth dirty white in colour.
	SE ±	0.509		
	CD at 5% level	1.53		

* Average of three replications
++++ Abundant sporulation
+++ Good sporulation
++ Moderate sporulation
\+ Poor sporulation
\- No sporulation

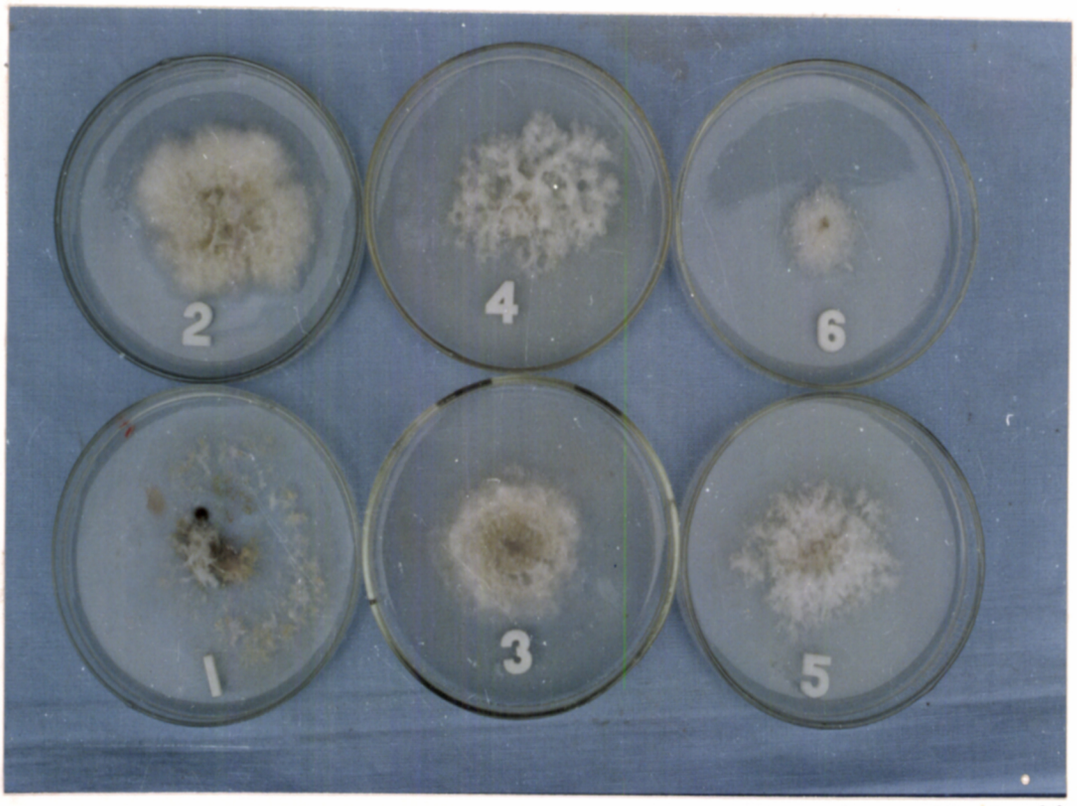
Table 3. Dry mycelial weight of Botryodiplodia theobromae Pat. in different liquid media after seven days of inoculation

Sr. No.	Media	*Mean dry mycelial weight (mg)
1.	Czepak's broth	410.00
2.	Coon's broth	311.00
3.	Sabourd's broth	448.33
4.	Richard's broth	480.00
5.	Potato dextrose broth	440.00
6.	Kirchoff's broth	460.00
7.	Nutrient broth	320.00
8.	Oat meal broth	325.00
	S.E. ±	2.06
	C.D. at 5% level	6.18

* Average of three replications

1. Czapek's agar
2. Coon's medium
3. Sabourd's medium
4. Richard's agar
5. Potato dextrose agar
6. Kirchoff's agar
7. Nutrient agar
8. Oat meal agar

Plate 8. Growth characters of Botryodiplodia theobromae Pat. of different media.



1. Glucose
2. Sucrose
3. Maltose
4. Lactose
5. Mannitol
6. Control

Plate 9. Growth characters of Botryodiplodia theobromae Pat. on carbon sources.

Among the liquid culture media tried, Richard's broth produced significantly higher dry mycelial weight to rest of liquid media i.e., 480 mg. It was followed by Kirchoff's broth (460.00 mg) and potato dextrose broth (440.00 mg). However, the lowest dry mycelial weight was recorded in Nutrient broth i.e. 320.00 mg.

4.5 Physiological studies

4.5.1 Effect of temperatures on growth and sporulation of Botryodiplodia theobromae Pat.

The result in respect of growth and sporulation of B.theobromae as influenced by different temperatures are presented in Table 4 and graphically shown in Fig.2.

From the data presented in Table 4, it is revealed that the pathogen could grow between the temperature ranged from 15°to 35°C. Maximum growth was observed at 30°C and 25° ± 2°C i.e.,88 mm at each temperature. There was no growth at 0°, 5°, 10° and 40°C temperature.

As regard to sporulation, it was observed that abundant sporulation was observed at 30°C, good sporulation at 25 ± 2°C and moderate sporulation at 20°C. However, poor sporulation was observed at 15°C and 35°C. There was no sporulation at 0°C, 5°C, 10°C and 40°C.

Table 4. Effect of temperature on growth and sporulation of Botryodiplodia theobromae Pat.

Sr. No.	Incubation temperature (°C)	* Mean colony diameter (mm)	Sporulation
1	0	-	-
2	5	-	-
3	10	-	-
4	15	28	+
5	20	42.5	++
6	25 ± 2	88.0	+++
7	30	88.0	++++
8	35	26.0	+
9	40	-	-

* Average of three replications

++++ Abundant sporulation

+++ Good sporulation

++ Moderate sporulation

\+ Poor sporulation

\- No sporulation

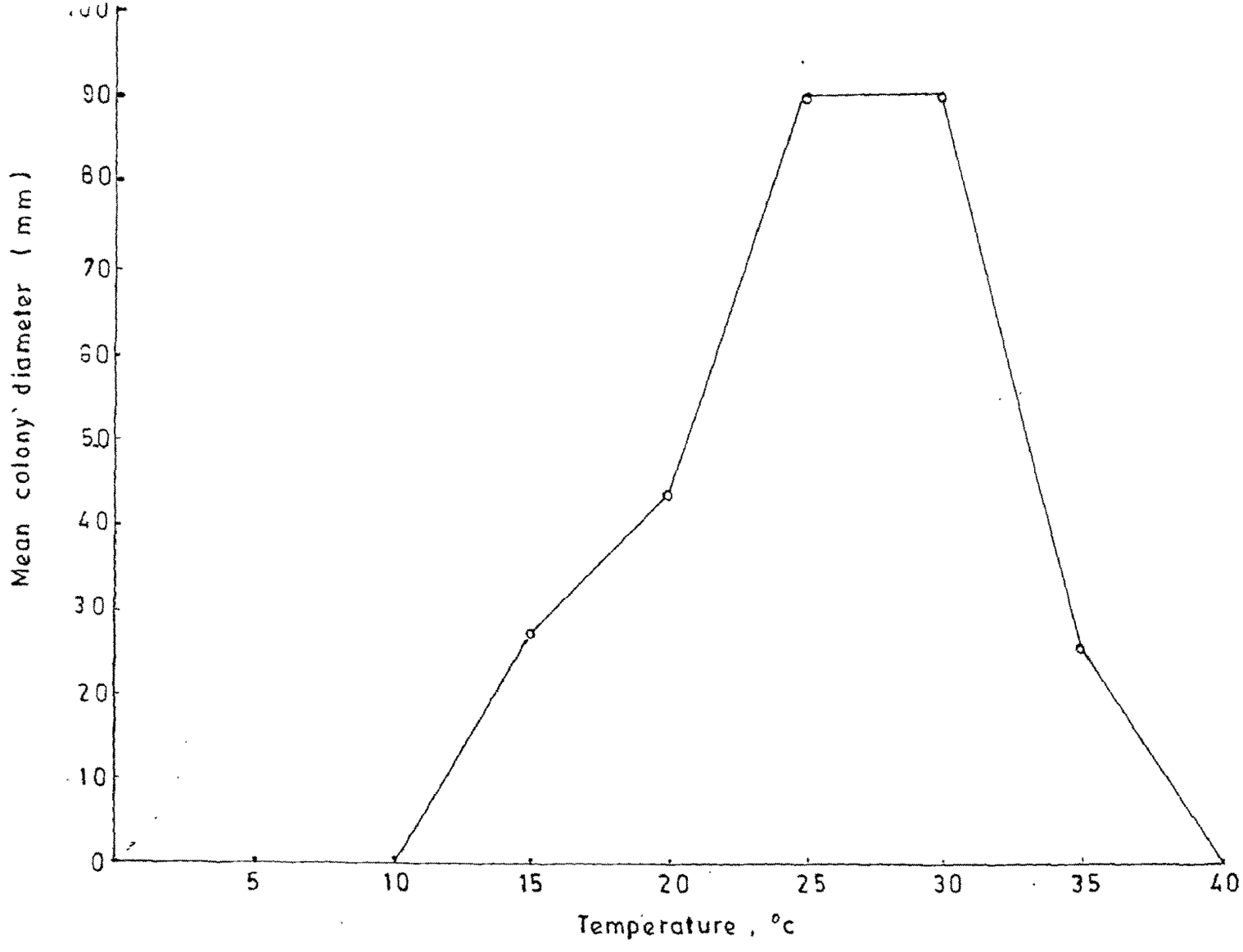


Fig. 2 Effect of temperatures on growth of Botryodiplodia th

Table 5. Utilization of carbon sources by Botryodiplodia theobromae Pat. on solid media after seven days of inoculation

Sr. No.	Source	*Mean colony diameter (mm)	Sporulation	Growth characters
1.	Glucose	80.5	++	Irregular colony with no entire margin, loose growth, dirty, white in colour but at centre buffy colour, poor growth.
2.	Sucrose	81.33	++++	Colony irregular, flat and moderate growth, dirty white in colour, loose mycelial growth.
3.	Maltose	62.00	+++	Colony circular with break margin, flat and submerged at margin, dirty white, moderate growth.
4.	Lactose	57.33	+++	Irregular colony with loose growth, white in colour, moderate growth.
5.	Mannitol	72.33	+++	Irregular colony with break margin, submerged growth, dirty in colour, moderate growth.
6.	Control	24.5	-	Circular colony with loose mycelium, poor growth.
	SE ±	0.735		
	CD. at 5%	2.26		

* Average of three replications

++++ Abundant sporulation

+++ Good sporulation

++ Moderate sporulation

\+ Poor sporulation

\- No sporulation

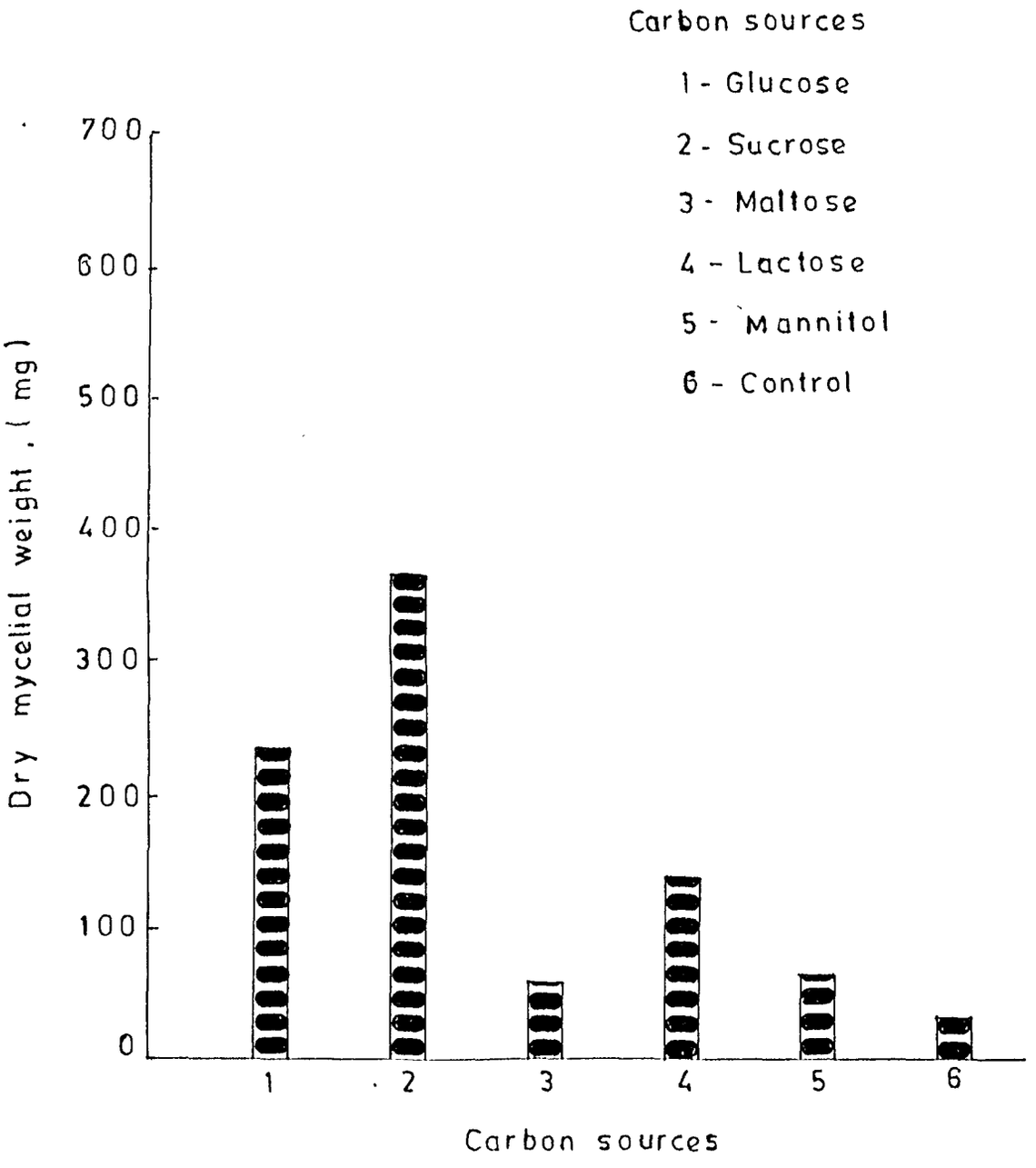


Fig.3 Effect of carbon sources on production of dry mycelial weight of Botryodiplodia theobromae Pat.

Table 6. Dry mycelial weight of Botryodiplodia theobromae Pat. in different carbon sources after seven days of inoculation

Sr. No.	Treatments	* Mean dry mycelial weight (mg)
1	Glucose	235
2	Sucrose	365
3	Maltose	55
4	Lactose	140
5	Mannitol	63.33
6	Control	30.00
	S.E.±	4.18
	C.D. at 5%	12.88

* Average of three replications

It is revealed from results that amongst the carbon sources sucrose significantly produced higher dry mycelial weight to rest of the carbon sources that is 365 mg. It was followed by glucose (235 mg) and lactose (140 mg) and were on par. However, lowest dry mycelial weight was recorded in maltose i.e. 55 mg. The control treatment produced only 30 mg dry mycelial weight of the pathogen.

4.5.3 Utilization of nitrogen sources

4.5.3.1 In solid media

The ability to pathogen i.e., B.theobromae to utilize different nitrogen sources was studied. The observ ations were recorded on solid media regarding colony diameter, sporulation and growth characters after seven days of inoculation which are presented in Table 7 and illustrated in Plate 10.

It is revealed from the results that the pathogen could utilize all types of nitrogen sources under study. Among the nitrogen sources tried for the characterisation of pathogen, potassium nitrate produced significantly good growth to rest of nitrogen sources i.e. 89.16 mm colony diameter and was followed by peptone (87 mm). Ammonium chloride produced least colony diameter of the pathogen that is 65.83 mm. However, control treatment showed 20.83 mm colony diameter.

As regard to sporulations, abundant sporulation was observed in sodium nitrate and potassium nitrate. Good

Table 7. Utilization of nitrogen sources by Botryodiplodia theobromae Pat. on solid media after seven days of inoculation

Sr. No.	Source	*Mean colony diameter (mm)	Sporulation	Growth characters
1.	Peptone	87	++	Colony circular, rainsed growth, white buffy colour, good growth.
2.	Ammonium chloride	65.83	++	Colony irregular, flat and poor growth with dirty white colour.
3.	Sodium nitrate	80.00	++++	Colony irregular, flat growth compact mycelium, good growth.
4.	Calcium nitrate	70.16	+++	Colony irregular, flat growth, white in colour at margin and buffy in the centre, moderate growth.
5.	Ammonium nitrate	83.00	+	Colony circular, raised growth white in colour at margin, buffy at centre, good growth.
6.	Potassium nitrate	89.16	++++	Colony circular with entire margin, raised growth, dirty white in colour, excellent growth
7.	Control	20.83	-	Colony irregular, submerged growth, gray in colour
	S.E. ±	0.350		
	C.D. at 5%	1.059		

\+ Average of three replications
++++ Abundant sporulation
+++ Good sporulation
++ Moderate sporulation
\+ Poor sporulation
\- No. sporulation

sporulation was observed in calcium nitrate. Moderate sporulation was observed in peptone and ammonium chloride. No sporulation was observed in control treatment.

4.5.3.2 In liquid media

The observations on dry mycelial weight of B.theobromae after seven days of inoculation are recorded and presented in Table 8. The results are also expressed graphically in Fig.4.

It is revealed from the results that the potassium nitrate used as nitrogen source produced significantly higher dry mycelial weight 375 mg followed by ammonium nitrate (91.66 mg) and sodium nitrate (74.33 mg). Lowest dry mycelial weight was recorded in peptone i.e. 47.66 mg. However, the control treatment produced only 27 mg of dry mycelial weight of the pathogen.

4.5.4 Effect of light on the growth of B.theobromae Pat.

In the present studies the fungus grew well and sporulate well under continuous fluoroscent light (850 lux) for 7 days, followed by room light (750 lux). Fluorescent light (850 lux) was the best treatment for good growth and abundant bicelled sporulation. Room light (750 lux) was the best for profuse growth, abundant single celled sporulation and good bicelled sporulation. Sunlight (3000 lux) recorded poor growth, abundant single celled and scanty bicelled spores. Whereas absence of light both in incubator and wrapped plates with

Table 8. Dry mycelial weight of Botryodiplodia theobromae Pat. in different nitrogen sources after seven days of inoculation

Sr. No.	Treatments	*Mean dry mycelial weight (mg)
1	Peptone	47.66
2	Ammonium chloride	56.00
3	Sodium nitrate	74.33
4	Calcium nitrate	64.33
5	Ammonium nitrate	91.66
6	Potassium nitrate	375.00
7	Control	27.00
	S.E. ±	1.97
	C.D. at 5%	5.96

* Average of three replications

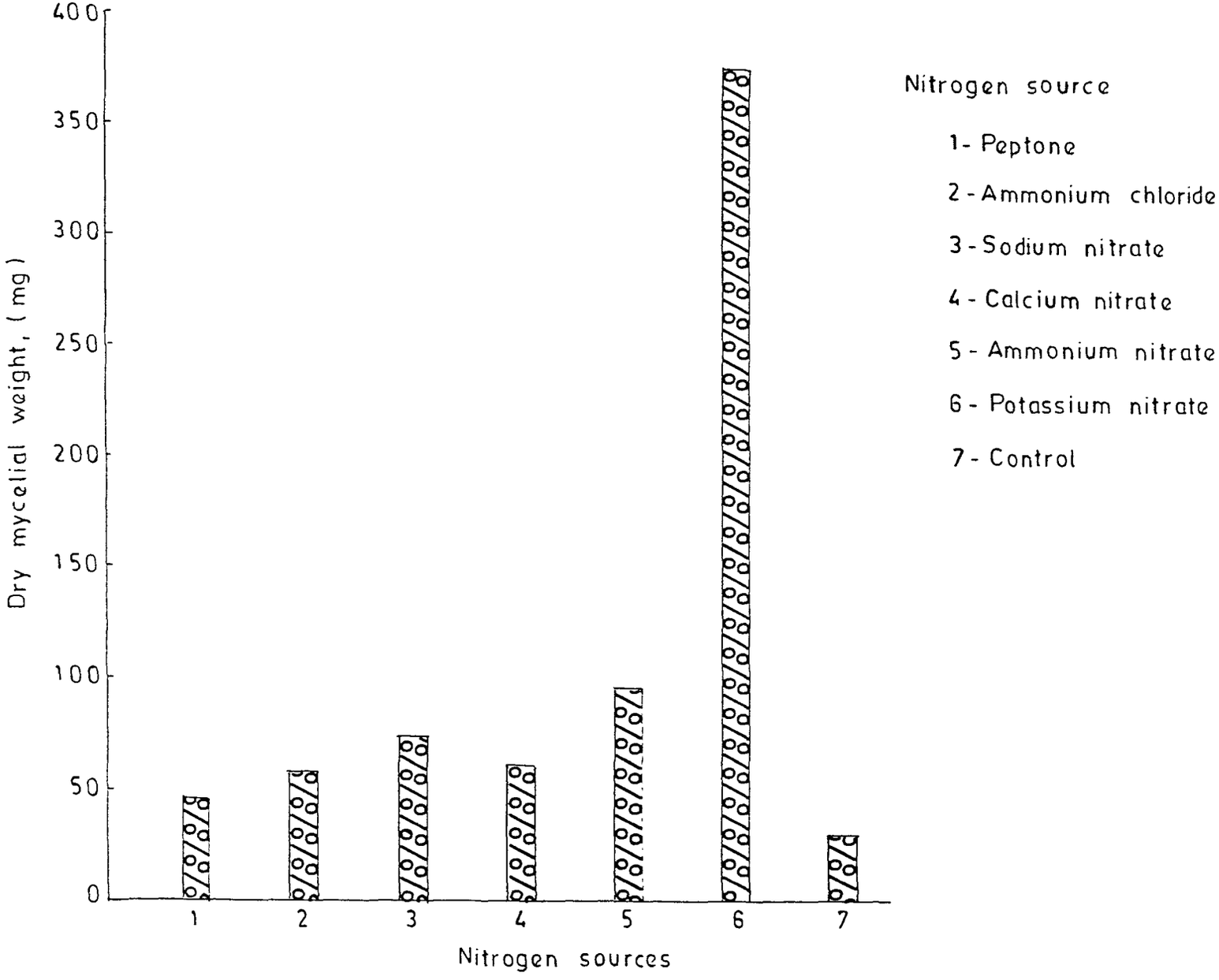
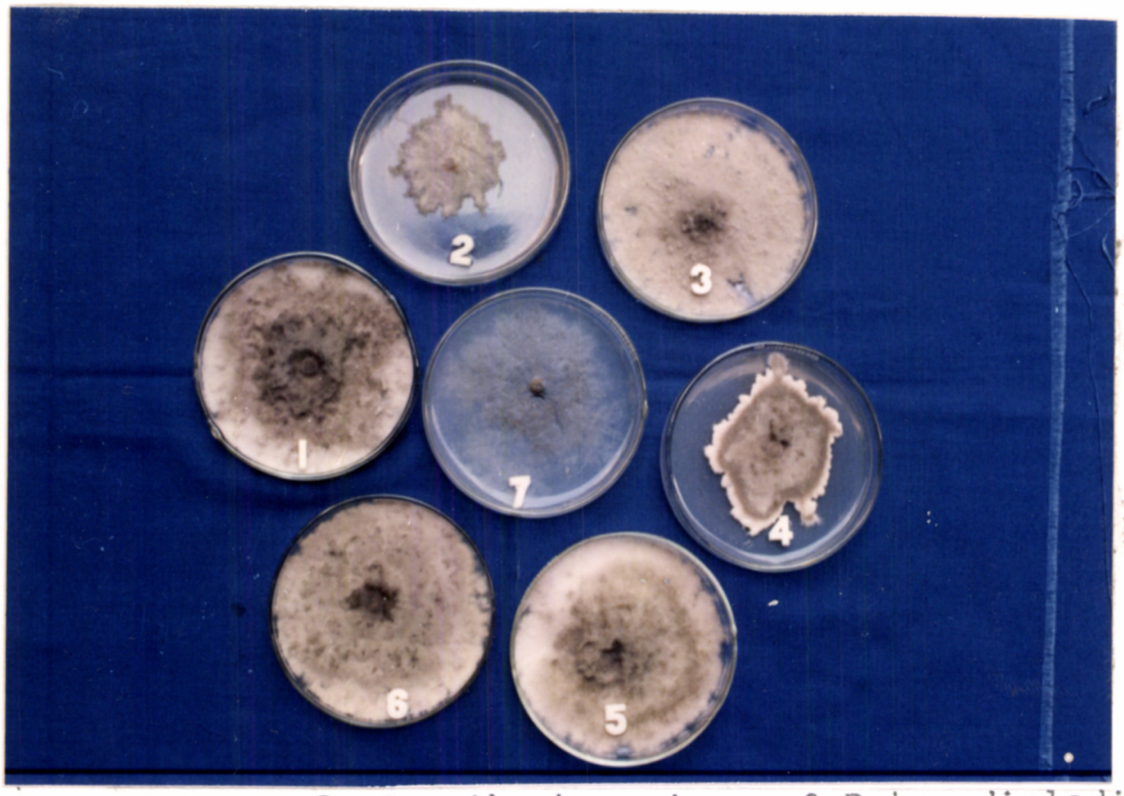


Fig. 4 Effect of nitrogen sources on production of dry mycelial weight of Botryodiplodia theobromae Pat.



1. Peptone
2. Ammonium chloride
3. Sodium nitrate
4. Calcium nitrate
5. Ammonium nitrate
6. Potassium nitrate
7. Control

Plate 10. Growth characters of Botryodiplodia theobromae Pat. on nitrogen sources.

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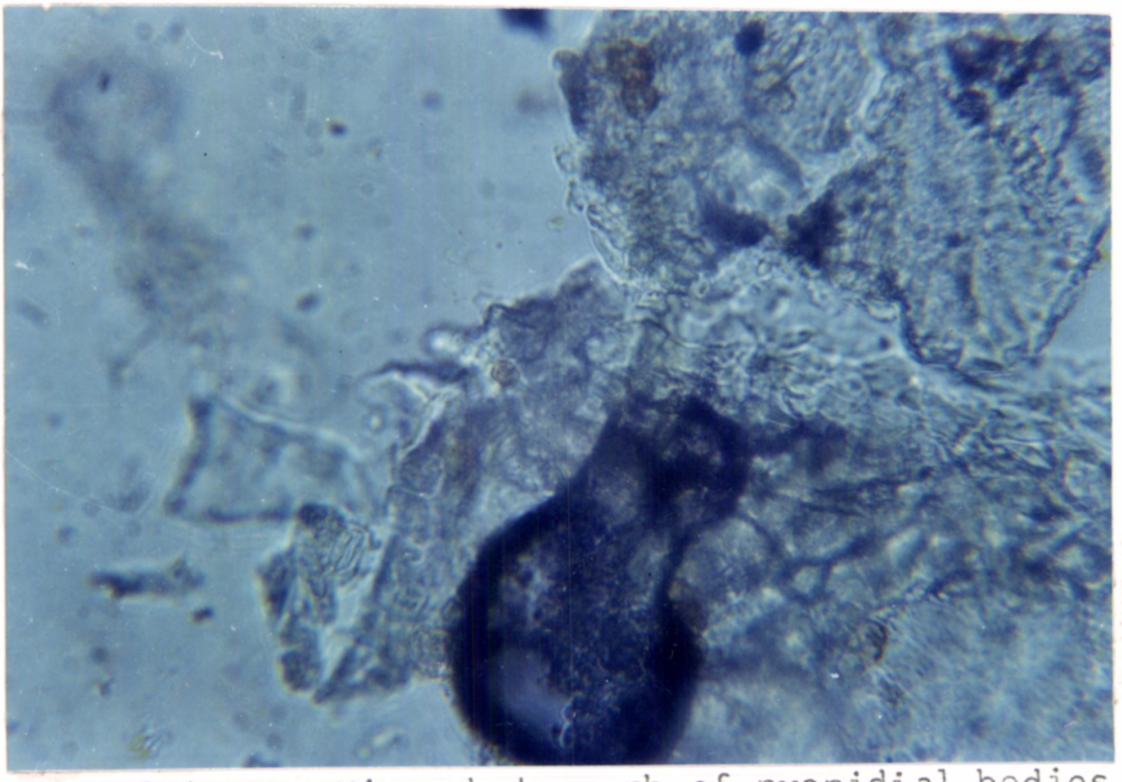


Plate 11. Microphotograph of pycnidial bodies on natural host (Mango) of Botryodiplodia theobromae Pat.

Table 9. Effect of different types of light on the growth and sporulation of B.theobromae after seven days of inoculation

Sr. No.	Types of light	Intensity of light (lux)	Growth characters		Sporulation	
			*Mean colony diameter (mm)	Growth	Single celled	Bicelled
1	Fluorescent	850	88.00	Good	++++	++++
2	Room light	750	88.00	Profuse	++++	+++
3.	Sunlight	3000	88.00	Poor	++++	+
4	Incubator (Partial light)	30	88.00	Profuse	++++	-
5	Darkness(Plates wrapped with black paper)	-	88.00	Profuse	++++	-

* Average of three replications

++++ Abundant sporulation

+++ Good sporulation

++ Moderate sporulation

\+ Poor sporulation

\- No sporulation

black papers produced profuse growth but failed to sporulate bicelled spores.

4.5.5 Occurrence of pycnidia of B.theobromae Pat.on twigs under natural conditions and under artificial conditions

Pycnidial bodies of B.theobromae on twigs under natural conditions were observed. Under artificial conditions B.theobromae appeared on twig. Profuse whitish growth and small black colour pycnidial bodies of B.theobromae on twigs of mango were observed.

4.6 Evaluation of fungicides against die back disease of mango (Botryodiplodia theobromae Pat.) in pot culture

4.6.1 Disease intensity

Mean length of infected area of disease twigs as recorded before first spraying is given in Table 10. The infected area of diseased twigs were recorded after first, second, third and fourth sprayings and same was analysed statistically and presented in Tables 11, 12, 13, 14, respectively.

4.6.1.1 Initial disease intensity

The initial length of infected area of disease on twigs in different plants of the fungicidal treatments was recorded on 10th day of inoculation. It ranged from 4.66 to 7.33 mm in the plants of different fungicidal treatments.

4.6.1.2 After first spraying

It is seen from the results (Table 11) that the treatment carbendazim (0.1%) was significantly superior to other fungicidal treatments and control in checking the disease intensity of die back disease. Next to these, thiophanate-methyl and captan were found effective in controlling the disease and were on par. Among all the fungicidal treatments carbendazim showed less infected area of the disease i.e., 8.33 mm as against 17.5 mm in the control treatment. It was followed by thiophanate-methyl and captan i.e., 11.5, and 12 mm, respectively.

Maximum per cent infection control was observed in the treatment carbendazim (52.4 per cent). It was followed by thiophanate-methyl and captan i.e., 34.28 and 31.42 per cent, respectively. Minimum per cent infection control (17.27%) was observed in the treatments copper oxychloride. The per cent infection control by other fungicides ranged from 17.14 to 22.85 per cent.

4.6.1.3 After second spraying

All the fungicidal treatments were found significantly superior to control treatment in checking the die back disease of mango (Table 12). The treatments carbendazim (0.1%), thiophanate-methyl (0.1%) and captan (0.2%) were found significantly superior to other fungicidal treatments and control in checking the disease intensity. Among all the

fungicidal treatments carbendazim showed less infected area of the disease i.e. 9.66 mm as against 26.5 mm in the control treatment. It was followed by thiophanate-methyl and captan i.e. 13.5 and 15.16 mm, respectively.

Maximum per cent infection control was recorded in the treatment carbendazim i.e. 63.54 per cent. It was followed by thiophanate-methyl and captan i.e., 49.05 and 42.79 per cent, respectively. Whereas minimum per cent infection control (25.81 per cent) was observed in the fungicidal treatment copper oxychloride. The per cent infection control by rest of the fungicides ranged from 30.18 to 42.79 per cent.

4.6.1.4 After third spraying

All the fungicidal treatments were found significantly superior to control treatment in controlling die back disease of mango (Table 13). The treatment carbendazim (0.1%) was found significantly superior to the rest of fungicides and control in checking the intensity of die back disease of mango. It was followed by thiophanate-methyl (0.1%) and captan (0.2%). Among all the fungicidal treatments, carbendazim showed less infected area of the disease i.e., 9.66 mm as against 39.5 mm in control treatment. It was followed by thiophonate-methyl and captan i.e., 13.5 and 17.5 mm, respectively.

Maximum per cent infection control was recorded in the treatment carbendazim i.e. 75.54 per cent. It was followed

by thiophanate-methyl and captan i.e. 65.82 and 55.69 per cent respectively whereas minimum per cent infection control (35.44%) was observed in copper oxychloride. The per cent infection control by rest of the fungicides ranged from 40.50 to 47.69 per cent.

4.6.1.5 After fourth spraying

It is seen from Table 14 that all fungicides used for the control of die back disease of mango were significantly superior to control treatment. The treatment carbendazim (0.1%) was found significantly superior to the rest of fungicides and control in checking the intensity of the die back disease of mango. It was followed by thiophanate-methyl (0.1%) and captan (0.2%). Next to these treatments benomyl (0.1%) and mancozeb (0.25%) were found effective and were on par. Among all the fungicidal treatments carbendazim showed less infected area of the disease i.e. 9.66 mm as against 48.5 mm in control treatment. It was followed by thiophanate-methyl and captan i.e. 13.5 and 17.5 mm, respectively.

Maximum per cent infection control was recorded in the treatment carbendazim i.e. 80.08 per cent. It was followed by thiophanate-methyl and captan i.e. 72.16 and 63.91 per cent, respectively. Minimum per cent infection control (37.11%) was observed in copper oxychloride. The per cent infection control by rest of the fungicides ranged from 37.11 to 43.29 per cent.

The replicationwise length of infected area after each spraying is represented in Table 15.

4.6.1.6 Effect of number of sprayings

The results of per cent infection control after each spraying are summarised in Table 16.

It is clear from the results that there was continuous increase in per cent disease control in all the fungicides used with increased number of sprayings. Among all the fungicides, maximum infection control i.e. 80.08 per cent was recorded in the fungicide carbendazim followed by thiophanate-methyl (72.16%) and captan (63.91%). The effect of the fungicides on per cent infection control is represented graphically in Fig.5.

4.7 Evaluation of fungicides against *Botryodiplodia theobromae* Pat. the cause of die-back disease of mango *in-vitro*

4.7.1 Solid media studies

It is revealed from the results presented in Table 17 that all fungicides showed inhibitory effect on growth of the pathogen as compared to control. The treatments benomyl (0.1%), carbendazim (0.1%), captan (0.2%), mancozeb (0.25%), thiophanate-methyl (0.1%) showed complete inhibition of growth of the fungus. It indicated that these fungicides were found highly effective in checking the growth of pathogen *B.theobromae* in solid media.

Table 10. Intensity of die back disease of mango before first spraying in different treatments

Sr. No.	Treatments	Length of infected area (mm)			Total	Mean
		RI	RII	RIII		
1	Aureofungin	6.0	6.5	8.0	20.5	6.83
2	Benomyl (Benomyl)	8.0	9.0	5.0	22.0	7.33
3	Captan (Captaf)	6.0	8.0	7.0	21.0	7.00
4	Carbendazim (Bavistin)	4.0	6.0	5.0	15.0	5.00
5	Copper oxychloride (Blitox)	5.0	5.0	4.0	14.0	14.66
6	Mancozeb (Indofil-M-45)	7.0	8.0	7.0	22.0	7.33
7	Thiophanate-methyl (Topsin-M-70)	8.0	7.0	6.0	21.0	7.00
8	Control	9.0	7.0	6.0	22.0	7.33

Table 11. Intensity of die back disease of mango after first spraying

Sr. No.	Treatments	Length of infected area (mm)			Total	Mean	Per cent infection control
		RI	RII	RIII			
1	Aureofungin	15.5	14.0	14.0	43.5	14.5	17.14
2	Benomyl (Benomyl)	14.5	12.5	13.5	40.5	13.5	22.85
3	Captan (Captaf)	10.5	13.0	12.5	36.0	12.0	31.42
4	Carbendazim (Bavistin)	8.5	8.25	8.25	25.0	8.33	52.4
5	Copper oxychloride (Blitox)	14.5	15.5	15.5	45.5	15.16	13.37
6	Mancozeb (Indofil-M-45)	14.5	13.5	14.0	42.0	14.00	20.00
7	Thiophanate-Methyl (Topsin-M-70)	11.0	12.0	11.5	34.5	11.5	34.28
8	Control	17.5	16.5	18.5	52.5	17.5	
	Total	106.5	105.25	107.75	319.5		
	S.E. ±					0.472	
	C.D. at 5%					1.414	

Table 12. Intensity of die back disease of mango after second spraying

Sr. No.	Treatments	Length of infected area (mm)			Total	Mean	Per cent infection control
		RI	RII	RIII			
1	Aureofungin	18.0	19.0	18.5	55.5	18.5	30.18
2.	Benomyl (Benomyl)	18.0	17.5	18.0	53.5	17.83	32.71
3	Captan (Captaf)	15.0	16.0	14.5	45.5	15.16	42.79
4	Carbendazim (Bavistin)	10.0	9.5	9.5	29.0	9.66	63.54
5	Copper oxychloride (Blitox)	20.0	19.5	19.5	59.0	19.66	25.81
6	Manozeb (Indofil-M-45)	18.0	17.5	19.5	55.0	18.33	30.83
7	Thiophanate-methyl (Topsin M-70)	13.0	14.0	13.5	40.5	13.5	49.05
8	Control	26.0	26.0	27.5	79.5	26.5	
	Total	138.0	139.0	140.5	417.5		
	S.E. ±					0.363	
	C.D. at 5% level					1.088	

Table 13. Intensity of die back disease of mango after third spraying

Sr. No.	Treatments	Length of infected area (mm)			Total	Mean	Per cent infection control
		RI	RII	RIII			
1	Aureogungin	24.0	23.5	23.0	70.5	23.5	40.50
2	Benomyl (Benomyl)	20.0	20.5	21.5	62.0	20.66	47.69
3	Captan (Captaf)	17.0	18.0	17.5	52.5	17.5	55.69
4	Carbendazim (Bavistin)	10.0	9.5	9.5	29.0	9.66	75.54
5	Copper oxychloride (Blitox)	24.5	24.5	27.5	76.5	25.5	35.44
6	Mancozeb (Indofil M-45)	21.5	22.5	22.0	66.0	22.0	44.30
7	Thiophanate-methyl (Topsin-M-70)	13.0	14.0	13.5	40.5	13.5	65.82
8.	Control	38.5	39.5	40.5	118.5	39.5	
	Total	168.5	172.0	175.0	515.5		
	SE. ±					0.485	
	CD. at 5% level					1.453	

Table 14. Intensity of die back disease of mango after fourth spraying

Sr. No.	Treatment	Length of infected area(mm)			Total	Mean	Per cent infection control
		RI	RII	RIII			
1	Aurofungin	27.0	27.5	28.0	82.5	27.5	43.29
2	Benomyl (Benomyl)	20.0	20.5	21.5	62.0	20.66	57.40
3	Captan (Captaf)	17.0	18.5	17.5	51.5	17.5	63.91
4	Carbendazim (Bavistin)	10.0	9.5	9.5	29.0	9.66	80.08
5	Copper Oxychloride (Blitox)	31.5	29.5	30.5	91.5	30.5	37.11
6	Mancozeb (Indofil M-45)	21.5	22.5	21.0	65.0	21.66	55.34
7	Thiophanate-methyl (Topsin-M-70)	13.0	14.0	13.5	40.5	13.5	72.16
8	Control	47.0	49.5	49.0	145.5	48.5	
	Total	187.0	191.0	190.5	568.5		
	S.E. ±					0.445	
	C.D. at 5% level					1.333	

Table 15. Replicationwise intensity of die back disease of mango of each spraying

Sr. No.	Treatments	Conc. %	Length of infected area (mm)			
			Replication-I			
			I	II	III	IV
1.	Aureofungin	0.05	15.5	18.0	24.0	27.0
2.	Benomyl (Benomyl)	0.1	14.5	18.0	20.0	20.0
3.	Captan (Captaf)	0.2	10.5	15.0	17.0	17.0
4.	Carbendazim (Bavistin)	0.1	8.5	10.0	10.0	10.0
5.	Copper oxychloride (Blitox)	0.25	14.5	20.0	24.5	31.5
6.	Mancozeb (Indofil-M-45)	0.25	14.5	18.0	21.5	21.5
7.	Thiophanate-methyl (Topsin-M-70)	0.1	11.0	13.0	13.0	13.0
8.	Control		17.5	26.0	38.5	47.0

Table 15 contd....

Replication - II

1	2	3	4	5	6	7
1.	Aureofungin	0.05	14.0	19.0	23.5	27.5
2.	Benomyl (Benomyl)	0.1	12.5	17.5	20.5	20.5
3.	Captan (Captaf)	0.2	13.0	16.0	18.0	18.0
4.	Carbendazim (Bavistin)	0.1	8.25	9.5	9.5	9.5
5.	Copper oxychloride (Blitox)	0.25	15.5	19.5	24.5	29.5
6.	Mancozeb (Indofil-M-45)	0.25	13.5	17.5	22.5	22.5
7.	Thiophanate-methyl (Topsin-M-70)	0.1	12.0	14.0	14.0	14.0
8.	Control		16.5	26.0	39.5	49.5

Table 15 contd.....

Replication - III

1	2	3	4	5	6	7
1.	Aureofungin	0.05	14.0	18.5	23.0	28.0
2.	Benomyl (Benomyl)	0.1	13.5	18.0	21.5	21.5
3.	Captan (Captaf)	0.2	12.5	14.5	17.5	17.5
4.	Carbendazim (Bavistin)	0.1	8.25	9.5	9.5	9.5
5.	Copper oxychloride (Blitox)	0.25	15.5	19.5	27.5	30.5
6.	Mancozeb (Indofil-M-45)	0.25	14.0	19.5	22.0	21.0
7.	Thiophanate-methyl (Topsin-M-70)	0.1	11.5	13.5	13.5	13.5
8.	Control		18.5	27.5	40.5	49.0

Table 16. Per cent infection control of die back disease of mango after each spraying

Sr. No.	Treatments	Conc. %	Spraying			
			I	II	III	IV
1.	Aureofungin	0.05	17.14	30.18	40.50	43.29
2.	Benomyl (Benomyl)	0.1	22.85	32.71	47.69	57.40
3.	Captan (Captaf)	0.2	31.42	42.79	55.69	63.91
4.	Carbendazim (Bavistin)	0.1	52.4	63.54	75.54	80.08
5.	Copper oxychloride (Blitox)	0.25	13.37	25.81	35.44	37.11
6.	Mancozeb (Indofil M-45)	0.25	20.0	30.83	44.30	55.34
7.	Thiophanate-methyl (Topsin M-70)	0.1	34.28	49.05	65.82	72.16

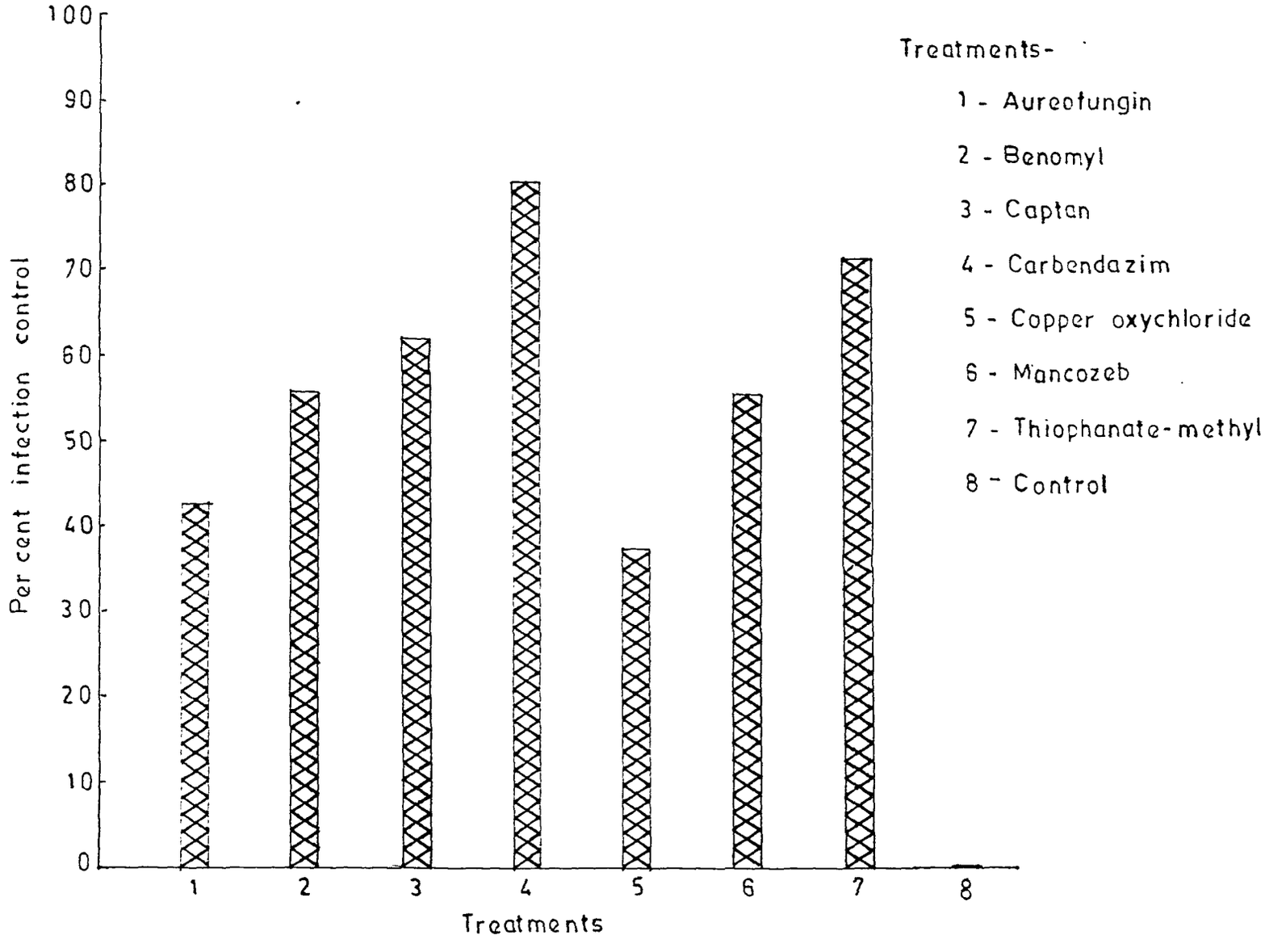


Fig. 5 Effect of fungicides for the control of die-back disease of mango caused by Botryodiplodia theobromae Pat. after fourth spraying.

Plate 12. Evaluation of fungicides against die back disease of mango (Botryodiplodia theobromae Pat.) in-vivo.

1. Aureofungin
2. Benomyl
3. Captan
4. Carbendazim
5. Copper oxychloride
6. Mancozeb
7. Thiophanate methyl
8. Control

Plate 13. Effect of fungicides on the growth and sporulation of Botryodiplodia theobromae Pat.the cause of die back disease of mango.

Table 17. Effect of fungicides on the growth and sporulation of Botryodiplodia theobromae Pat. the cause of die back disease mango

Sr. No.		Conc. %	*Mean colony diameter (mm)	Per cent inhibition	Sporulation
1.	Aureofungin	0.05	8.16	90.36	+
2.	Benomyl (Benomyl)	0.1	-	100.00	-
3.	Captan (Captaf)	0.2	-	100.00	-
4.	Carbendazim (Bavistin)	0.1	-	100.00	-
5.	Copper oxychloride (Blitox)	0.25	22.0	74.01	+
6.	Mancozeb (Indofil-M-45)	0.25	-	100.00	-
7.	Thiophanate-methyl (Topsin-M-70)	0.1	-	100.00	-
8.	Control	-	84.66	-	++++
	S.E.±		99.60		
	C.D. at 5% level		332.36		

* Average of three replications

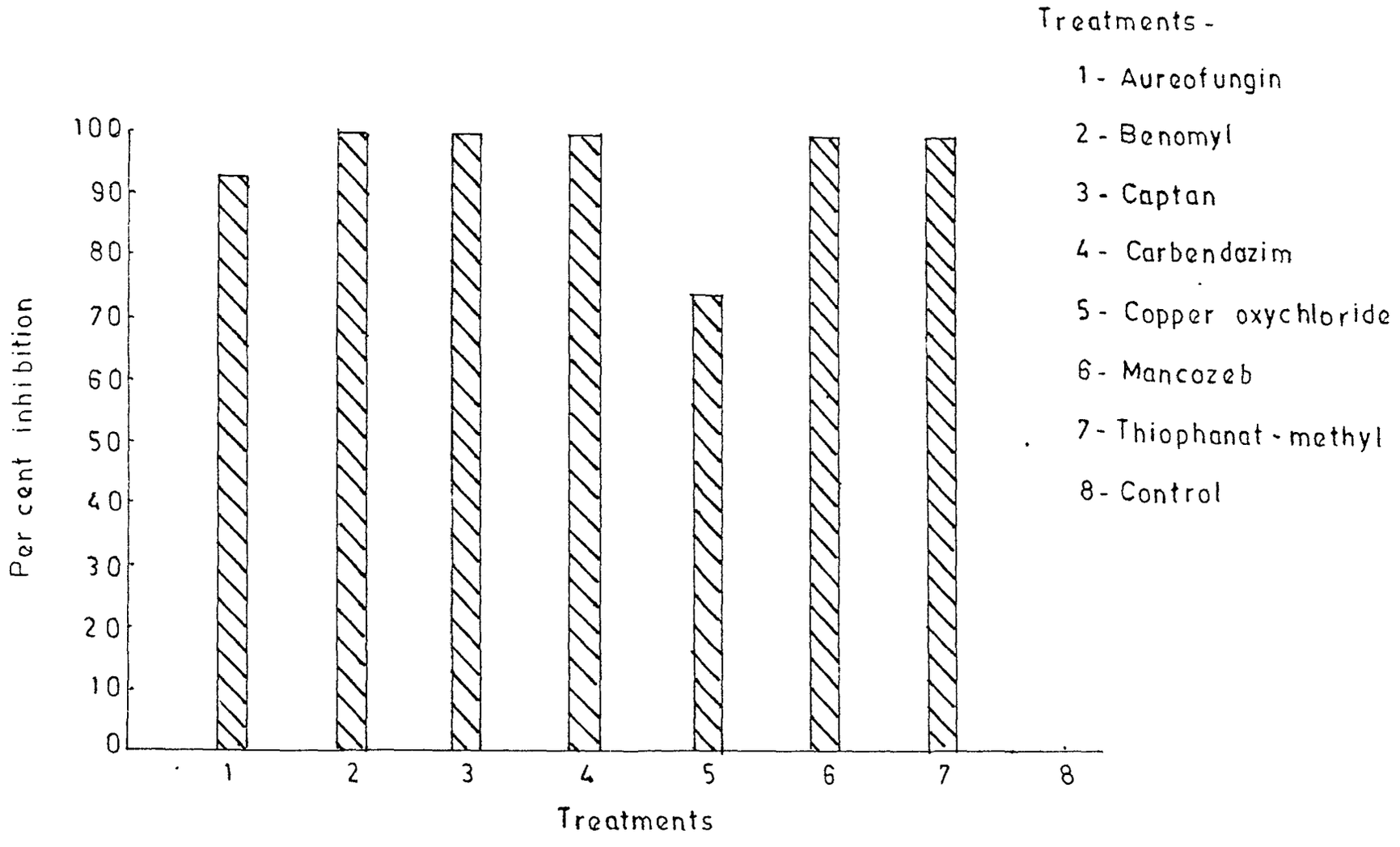


Fig. 6 Effect of fungicides on the inhibition of growth of Botryodiplodia theobromae Pat. on solid media.

Table 18. Effect of fungicides on the production of dry mycelial weight of Botryodiplodia theobromae Pat. the cause of die back disease of mango

Sr. No.	Treatments	Conc. %	*Mean dry mycelial wt (mg)	Per cent inhibition
1.	Aureofungin	0.05	37.33	90.90
2.	Benomyl (Benomyl)	0.01	-	100.00
3.	Captan (Captaf)	0.2	-	100.00
4.	Carbendazim (Bavistin)	0.1	-	100.00
5.	Copper oxychloride (Blitox)	0.25	160.33	60.95
6.	Mancozeb (Indofil-M-45)	0.25	-	100.00
7.	Thiophanate-methyl (Topsin-M-70)	0.1	-	100.00
8.	Control	-	410.66	-
	SE. ±		0.404	
	CD, at 5% level		1.348	

* Average of three replications

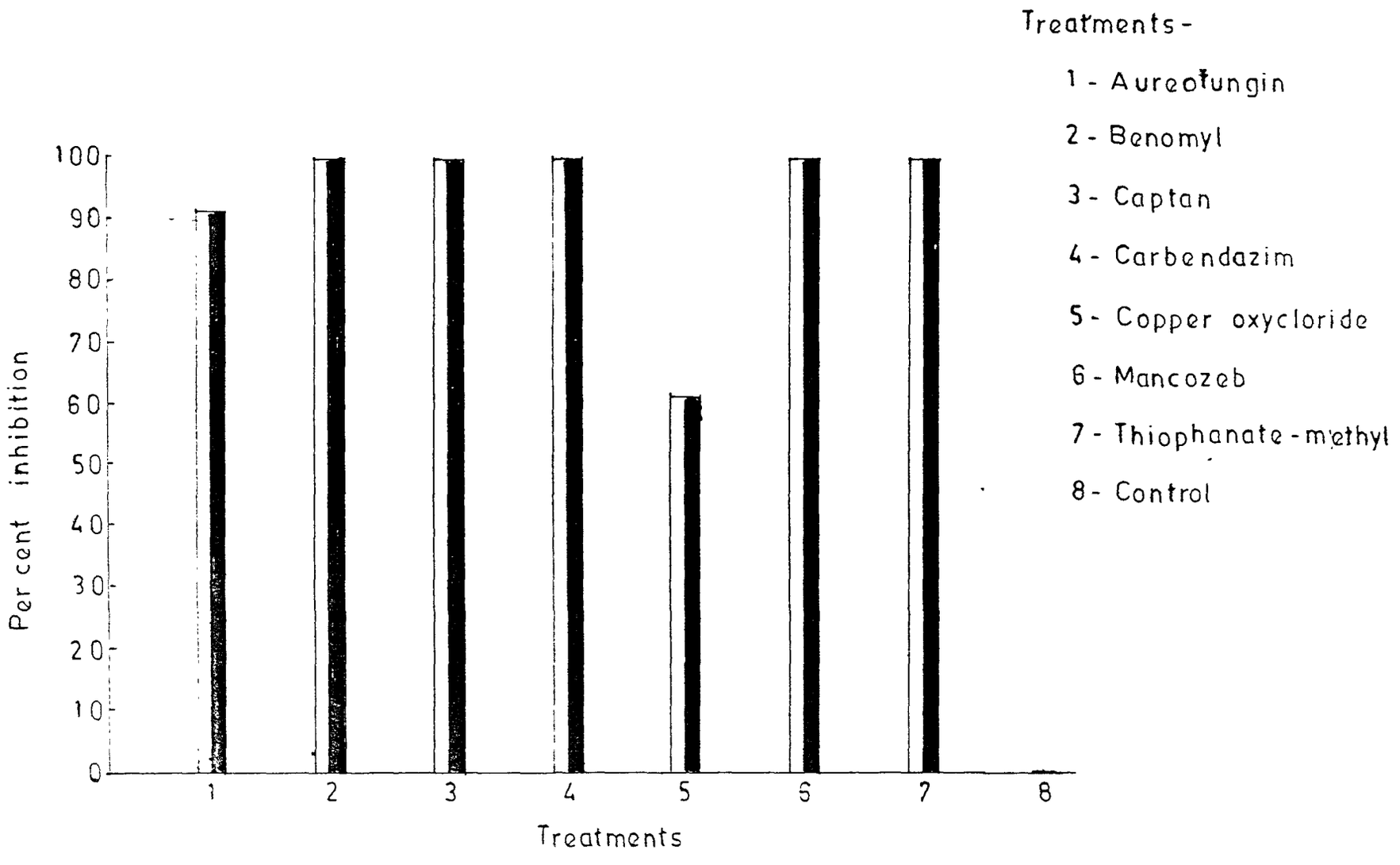
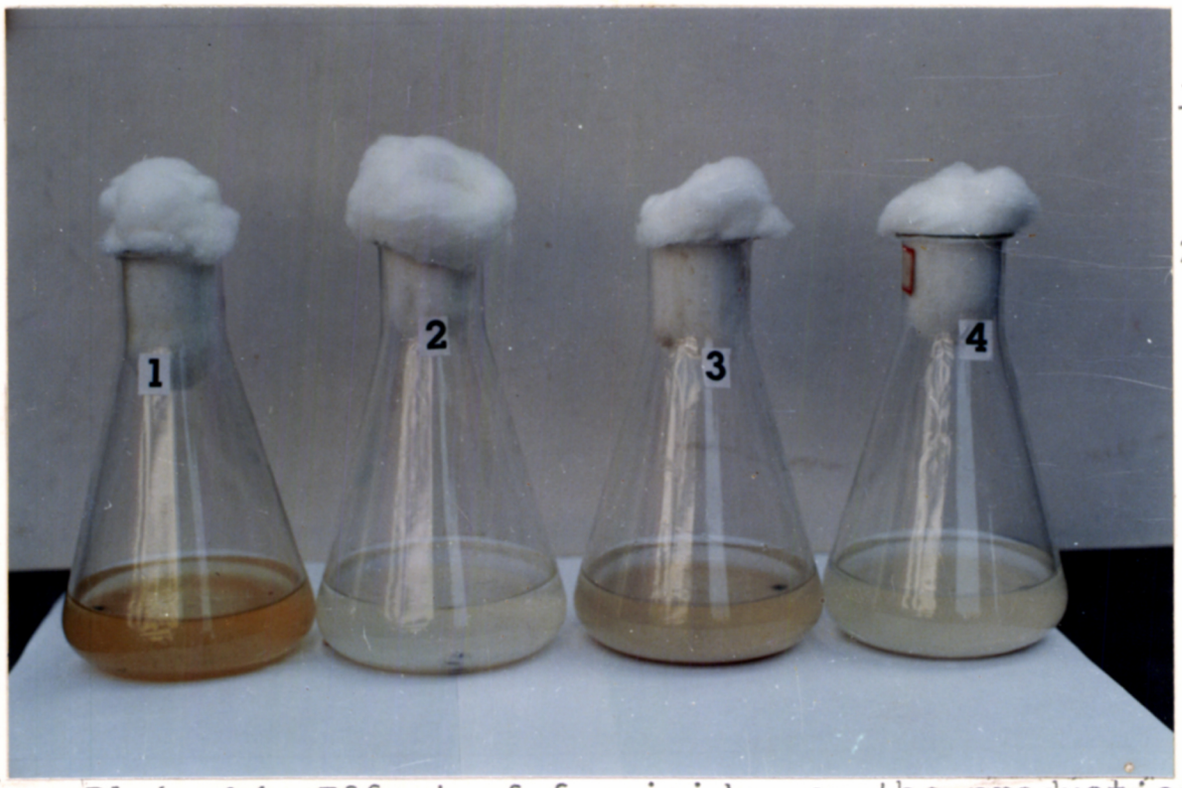


Fig. 7 Effect of fungicides on the production of dry mycelial weight of Botryodiplodia theobromae Pat.

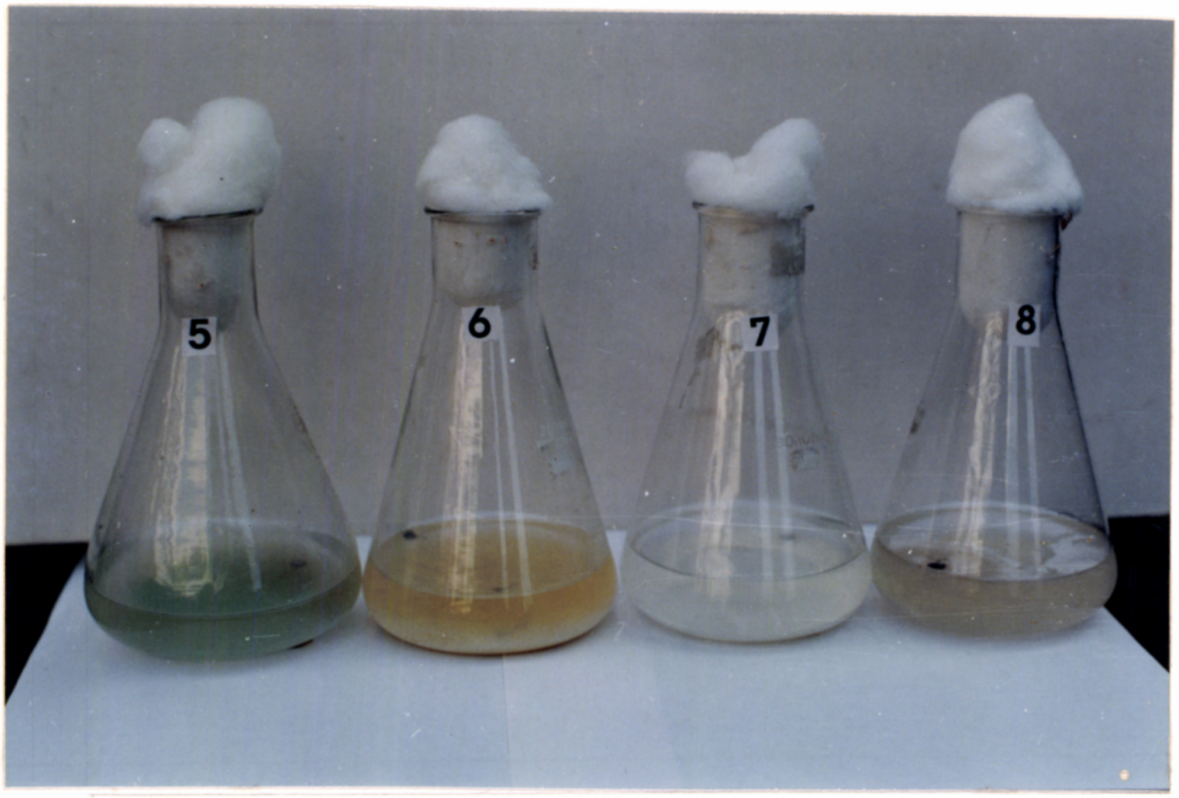
A



1. Aureofungin
2. Benomyl
3. Captan
4. Carbendazim

Plate 14. Effect of fungicides on the production of dry mycelial weight of Botryodiplodia theobromae Pat. the cause of die back disease of mango.

B



5. Copper oxychloride
6. Mancozeb
7. Thiophanate-methyl
8. Control

Plate 15. Effect of fungicides on the production of dry mycelial weight of Botryodiplodia theobromae Pat. the cause of die back disease of mango.

As regard per cent inhibition of dry mycelial weight of B.theobromae, the benomyl, carbendazim, captan, mancozeb, thiophanate-methyl showed 100 per cent inhibition in production of dry mycelial weight. However, the fungicides aureofungin showed 90.90 per cent inhibition whereas copper oxychloride showed 60.95 per cent inhibition in production of dry mycelial weight.

4.8 Screening of germplasm of mango against die back disease under natural conditions of disease development

The object of varietal screening was to find the resistant source to die back disease which could be an economic method for the control of this disease.

The varietal reaction of 76 mango varieties against die back disease was recorded under natural conditions of disease development. The results are presented in Table 19 and summarised in Table 20.

It is seen from the results that out of 76 varieties screened only one variety namely Bombay hutto was observed to be resistant under natural conditions of disease development. From the rest of the varieties, 58 varieties of mango showed moderately susceptible reaction and 17 varieties showed susceptible reaction. None of the variety was observed to be highly susceptible under natural conditions of disease development.

Table 19. Summary table on varietal screening of germplasm against die back disease of mango caused by Botryodiplodia theobromae Pat.

Grade	Percentage disease incidence (PDI)	Disease reaction	Number of varieties
1.	1-5	R	1
2.	6-20	MS	58
3.	21-50	S	17
4.	51-100	HS	-
Total	-	-	76

Where as :

R = Resistant

MS = Moderately susceptible

S = Susceptible

HS = Highly susceptible

Table 20. Screening of germplasm of mango against die back disease caused by Botryodioplodia theobromae Pat. under natural conditions of disease development

Sr. No.	Name of the variety	Percentage disease incidence (PDI)	Disease reaction
1	2	3	4
1.	Bombay yellow	15.96	MS
2.	Bombay deshera	13.15	MS
3.	Bombay rizode	22.35	S
4.	Salem	19.8	MS
5.	Golden selection	25.56	S
6.	Safed lucknow	19.04	MS
7.	Swamini	15.93	MS
8.	Sardar	15.84	MS
9.	Safeda	22.64	S
10.	Badami model	14.18	MS
11.	Villai columban	17.12	MS
12.	Ben choda	20.09	S
13.	Dilpasand	14.69	MS
14.	Jahangir	12.2	MS
15.	Hemsagar	11.02	MS
16.	Vasibadam	20.84	S
17.	Malgoa	15.09	MS

Contd....

Table 20 contd....

1	2	3	4
18.	Amelpur	16.5	MS
19.	Bensai	20.97	S
20.	Misteri	21.23	S
21.	Malda	7.76	MS
22.	Cowasji Patel	14.6	MS
23.	Rajmane	10.6	MS
24.	Amini	11.74	MS
25.	Belkhas	14.64	MS
26.	Kelakende	8.51	MS
27.	Paiposha	8.96	MS
28.	Chittoor	21.25	S
29.	Krishn bhog	15.82	MS
30.	Kervinda	18.60	MS
31.	Bombay butto	3.65	R
32.	Nartio	7.10	MS
33.	Fazli kalam	16.72	MS
34.	Faz li long	16.22	MS
35.	Ambe huide	10.14	MS
36.	Seedling	15.53	MS
37.	Surekha	8.23	MS

Table 20 contd....

1	2	3	4
38.	Peter pasand	12.36	MS
39.	Chittoormalgoba	21.09	S
40.	Puthi	16.16	MS
41.	Sheshmani	23.78	S
42.	Ambe lawi	26.04	S
43.	Totapuri	18.82	MS
44.	Rashpuri	15.48	MS
45.	Salem badam	9.04	MS
46.	Sindori	27.05	S
47.	Keshar	19.55	MS
48.	Samer basti alibag	10.64	MS
49.	Dashhari	11.69	MS
50.	Neelum	15.06	MS
51.	Aminkhan	10.5	MS
52.	Vanraj	12.74	NS
53.	Lang ra	10.01	MS
54.	Barsha	13.33	MS
55.	Goa mankur	17.28	MS
56.	Alphonso (Hapus)	24.81	S

Contd...

Table 20 contd.....

1	2	3	4
57.	Rajapuri	11.11	MS
58.	Pairi	14.02	MS
59.	Dadumiya	17.12	MS
60.	Dudhpedha	14.86	MS
61.	Fernanding	22.11	S
62.	Benarashi langada	19.45	MS
63.	Vishwanath mukha	14.51	MS
64.	Hybrid 7/1	14.05	MS
65.	Hybrid 3/7	22.75	S
66.	Hybrid 2/4	19.62	MS
67.	Chinasuveranrecha	17.46	MS
68.	Kitta beni	12.82	MS
69.	Neelgoa	18.65	MS
70.	Banet alphonso	21.82	S
71.	Allseason mango	23.43	S
72.	Amrapali	19.5	MS
73.	Mallika	14.64	MS
74.	Sunderaj	11.92	MS
75.	Sented pairi	14.11	MS
76.	Badami	19.89	MS

Discussion

5. DISCUSSION

Mango is one of the most important fruit crops in Maharashtra. The die back disease of mango caused by Botryodiplodia theobromae Pat. was observed on Alphonso during 1992. Studies on isolation and pathogenecity of pathogen, symptomatology of the disease, morphological, cultural and physiological characteristics of the pathogen, evaluation of fungicides in-vivo and in-vitro against the disease, screening of germplasm of mango against this disease were carried out. The results obtained from the investigations are discussed hereunder :

5.1 Isolation and pathogenecity

The isolation of the pathogen was made from infected twigs of mango, and pathogenecity was proved by artificial inoculation method. Reisolation was made from the infected plant parts and was identical to the original one. The casual fungus was identified as Botryodiploida theobromae Pat. The results are in close agreement with the following scientists. Botero (1972) proved the pathogenecity of Botryodiplodia theobromae causing die back disease of Ceropia tessani. Om Prakash and Raoof (1979) isolated B.theobromae causing die back disease of mango from green living twigs and branches showing discoloured cankers. They also proved the pathogenicity on mango seedling by injury method. A small slit was made with the help of sterlized

knife at node adjacent to the bud and mycelium alongwith the medium inserted in the slits. Trindade and Gasparotto (1982) isolated B.theobromae from infected plant of rubber and proved its pathogenicity by inoculation.

5.2 Symptomatology

Symptoms were characterised by wilting of branches and twigs, particularly of trees followed by complete defoliation of leaves and gave the tree on appearance as if it was scorched by fire. Colouring and darkening of bark at a certain distance from the tip was the external evidence of the disease. Such dark patches were seen on young green twigs and were hardly distinguishable in older branches. The bark was discoloured at several points when the dark lesion increased affecting the mid rib. The upper leaves lost their healthy green colour and gradually turned brown. This was followed by browning of whole leaf accompanied by the upward rolling of the margin. Finally the twig was found shrivelled which was an advance stage of disease. Internal browning in wood tissue was observed in slitting alongwith axis.

The symptoms observed in the present studies were more or less similar to those described by following scientists. Rath (1978) described the symptoms of die back disease of mango caused by B.theobromae The disease manifests three distinct symptoms, namely wither tip, twig blight and die back

disease which they considered as separate diseases. In the die back phase, large branches and the basal trunk developed bark cankers and gummy exudation. Eventuallyeven large branches of the entire tree died. The symptoms observed during the present investigation were similar to those described by Om Prakash and Raoof (1979) and Burhan (1987) for die back disease of mango caused by Botryodiplodia theobromae.

5.3 Morphology and spore germination

5.3.1 Morphology

The fungal pathogen B.theobromae produced cottony white mycelium which was irregularly branched, septate, primarily hyaline but with age turned olive grey to olive black in colour within seven days. Pycnidial bodies were formed in six days. Pycniospores were hyaline at early stages of growth and turned brownish. They were single celled primarily and became bicelled at maturity within 19 days of inoculation. They were oval to oblong granular at early stages. The bicelled pycniospores were 11.16-15.11 um in length and 7.39-11.75 um in breadth. These results are more or less in agreement with those reported by Sherkar (1979), Wardlaw (1932), Om Prakash and Raoof (1979), Gadage (1984).

5.3.2 Spore germination

Germination of single celled spores of Botryodiplodia theobromae was observed within eight hours in different

media and recorded on 10th hours of inoculation. Maximum germination was obtained in host leaf extract i.e., 99 per cent, followed by one per cent sugar solution,(94%) distilled water (84%), tap water (85%) and sterile water (83%). These results are more or less in close agreement with Gadage (1984) who reported maximum germination of B.theobromae in grape host extract (100 per cent). It was followed by one per cent sugar solution (91%) and tap water (81%).

5.4 Cultural studies

5.4.1 Solid media studies

Cultural characters of B.theobromae Pat. were studied on different media. Among the solid culture media tried for characterisation, Richard's agar (90.00 mm), Sabourd's agar (90.00 mm), and potato dextrose agar (79.17 mm) produced significantly good growth to rest of culture media. Moreover, abundant sporulation was observed on Richard's agar and potato dextrose agar. These results are more or less in close agreement with following research workers. D'Souza (1963) reported that among the synthetic and non synthetic media tried for growth and sporulation of B.theobromae Richard's agar and potato dextrose agar supported good growth. Patil (1966) reported that Sabourd's agar, Kirchoff's media, Richard's agar were best for growth and sporulation of B.theobromae. Sherkar (1979) showed that among the various

media tried, significantly superior growth and sporulation of B.theobromae was observed on potato dextrose agar. Gadage (1984) pointed out that among the synthetic media, Richard's agar support best growth and sporulation of B.theobromae.

5.4.2 Liquid media studies

During the present investigation Richard's broth produced significantly higher dry mycelial weight of B.theobromae to rest of the liquid media i.e., 480 mg. It was followed by Kirchoff's broth (460 mg) and Sabourd's medium (448.33 mg). These results are similar to those reported by Shinde (1988).

5.5 Physiological studies

5.5.1 Effect of temperature on growth and sporulation of B.theobromae

Maximum growth of this pathogen was observed at 25 ± 2°C and 30°C i.e., 88 mm colony diameter each. There was no growth at 0°, 5°, 10° and 40°C temperature. As regard to sporulation it was abundant at 30°C, whereas good sporulation was recorded at 25 ± 2°C. There was no sporulation at 0°, 5°, 10° and 40°C temperature. These results are more or less similar with the findings of following scientists. Srivastava and Tandon (1969) reported that B.theobromae of guava thrive between 15 and 35°C temperature. It did not grow at 10 and 40°C. The optimum temperature for growth and

pycnidial production was 25°C but conidial germination was maximum at 30°C. Verma and Singh (1970) reported that good growth and sporulation of the pathogen B.theobromae of die back disease of mango was at 27 to 30°C temperature.

5.5.2 Utilization of carbon source

5.5.2.1 In Solid Media Studies

Among the carbon source tried for characterisation of the B.theobromae sucrose and glucose significantly produced good growth to rest of the sources tried i.e., 81.33 mm and 80.5 mm, respectively and they were on par with each other. It was followed by mannitol i.e., 72.33 mm colony diameter. Abundant sporulation of this pathogen was observed on sucrose, whereas good sporulation was observed on maltose, lactose and mannitol. These results in respect of sucrose are in agreement with Gupta (1977), Gadage (1984). However, the results in respect of glucose are in agreement with Srivastava and Tandon (1968).

5.5.2.2 Liquid media studies

Among the carbon sources tried sucrose produced significantly higher dry mycelial weight of B.theobromae to rest of carbon sources i.e. 365.0 mg. It was followed by glucose (235.0 mg) and lactose (140 mg) and they were on par to each other. The results obtained during present investigations are more or less similar to those reported by

Shinde (1988), who showed that sucrose was significantly superior in production of higher dry mycelial weight of B.theobromae from rose than in glucose, fructose, cellulose, maltose, starch, sorbitol, lactose, galactose and xylose.

5.2.3 Utilization of nitrogen sources

5.2.3.1 Solid media studies

Among the nitrogen sources tried for the characterisation of the B.theobromae potassium nitrate produced significantly good growth to rest of nitrogen sources i.e., 89.16 mm colony diameter and was followed by Peptone (87 mm). Moreover, abundant sporulation was observed in sodium nitrate and potassium nitrate. However, good sporulation was observed in calcium nitrate. These results are more or less in close agreement with following workers. Patil (1966) showed good growth and sporulation of B.theobromae of grape on potassium nitrate and sodium nitrate. Srivastava and Tandon (1968) reported good growth of B.theobromae of guava on sodium nitrate. Sherkar (1979) showed good growth and sporulation of B.theobromae on potassium nitrate and sodium nitrate.

4.5.3.2 Liquid media studies

Among the nitrogen sources potassium nitrate produced significantly higher dry mycelial weight of B.theobromae to rest of nitrogen sources i.e. 375 mg. It was followed by ammonium nitrate (91.66 mg) and sodium nitrate (74.33 mg). The results obtained during present investigations are more

or less similar to those reported by Shinde (1988) who stated that potassium nitrate was significantly superior in production of higher dry mycelial weight of B.theobromae of rose and was followed by sodium nitrate.

4.5.4 Effect of light on the growth of Botryodiplodia theobromae Pat.

In the present studies the fungus grew well and sporulated well under continuous fluorescent light (850 lux) for 7 days, followed by room light (750 lux). Fluorescent light (850 lux) was the best treatment for good growth and abundant bicelled sporulation, as well as single celled spores. Whereas, room light (750 lux) was the best for profuse growth, abundant single celled sporulation and good bicelled sporulation. Sunlight (3000 lux) recorded poor growth, abundant single celled and scanty bicelled spores. Whereas absence of light both in incubator and wrapped plates with black paper produced profuse growth and moderate single celled spores. These results are more or less in close agreement with Ekundayo and Haskins (1969) who found B.theobromae produced abundant pycnidial bodies in fluorescent light but not in darkness. According to Alsodura (1970) 850 lux or 400 to 520 lux light was necessary for production of pycnidia in the culture of B.theobromae.

5.2.4 Occurrence of pycnidia of B.theobromae on twigs under natural conditions and under artificial conditions

Pycnidial bodies of B.theobromae on twigs of mango were observed under natural condition. Profuse whitish growth and small black colour pycnidial bodies of B.theobromae on twigs of mango appeared under artificial conditions i.e., in the incubator in the laboratory. Similar type of production of pycnidia was reported by Gadage (1984) on grape twigs.

5.6 Evaluation of fungicides against die back disease of mango caused by Botryodiplodia theobromae Pat. in-vivo

Seven fungicides were evaluated in pot culture studies against this disease. All fungicides were significantly superior to control after final spraying in controlling this disease. The treatment carbendazim (0.1%) was found significantly superior to rest of fungicides and control in checking the length of infected area on mango twig. It was followed by thiophanate-methyl (0.1%) and captan (0.2%). Among all the fungicides, carbendazim showed less infected area on mango twigs i.e., 9.66 mm as against 48.5 mm in the control treatment. It was followed by thiophanate-methyl and captan i.e. 13.5 and 17.5 mm, respectively. Among the all fungicides the treatment carbendazim showed highest percentage infection control i.e., 80.08 per cent and was followed by thiophanate-methyl, captan i.e. 72.16 and 63.91 per cent, respectively.

These results are more or less in close agreement with the following workers. Sivaprakasam (1976) reported that out of different fungicides used against Tapioca sett rot caused by D.natalensis, bavistin was most effective. Shirkar (1979) showed that out of 8 fungicides used against B.theobromae on pomegranate in field, benlate was found to be most superior in reducing percentage disease index (PDI) and increasing the percentage disease control. Dithane-Z-78, bavistin, dithane M-45 were next best fungicides.

Rawal and Ullasa (1984) reported that out of different fungicides used against B.theobromae bavistin (0.1%) and Jkstein (0.1%) were most effective.

Gadage (1984) used 11 fungicides against B.theobromae causing fruit rot of grapevine. Most of the fungicides were found effective against this pathogen. The fungicides, aureofungin, bavistin, benlate, mancozeb, copper oxychloride and ziride were found significantly superior to control in checking the pathogen.

5.7 Evaluation of fungicides against Botryodiplodia theobromae Pat., in-vitro the cause of die back disease of mango

In in-vitro, the efficacy of different fungicides were studied to identify the effective fungicides which can inhibit the growth and sporulation of Botryodiplodia theobromae.

In solid media studies, benomyl (0.1%), carbendazim (0.1%), captan (0.2%), mancozeb (0.25%) thiophanate-methyl (0.1%) showed complete inhibition of the growth of the fungus. It indicated cent per cent inhibition of the pathogen. Among the rest of treatments aureofungin (0.05%) and copper oxychloride (0.25%) were found significantly superior to control. These treatments were on par with each other in checking the growth of the pathogen. Least sporulation was observed in aureofungin (0.05%) and copper oxychloride (0.25%).

The results obtained during present investigations were more or less in agreement with those of the following workers. Chin (1967) reported that the fungicide dithane M-45 was completely inhibitory to the growth and sporulation of Botryodiplodia theobromae fungus obtained from fruit rot of banana.

Srivastava and Tandon (1971) reported that the fungicide captan was completely inhibitory to the growth and sporulation of B.theobromae the cause of fruit rot of guava.

More (1976) showed that fungicides benlate, blitox, dithane M-45 and difolatan were found to be most effective in inhibiting growth and sporulation of B.theobromae the cause of rot of grape berries.

Sherkar (1979) showed that benlate completely inhibited the growth of B.theobromae the cause of fruit rot of pomegranate. However, bavistin and dithane M-45 showed the inhibition 58.38 and 62.55 per cent, respectively.

Gadage (1984) showed that out of 11 fungicides, aureofungin, bavistin and benlate completely inhibited growth and sporulation of the fungus B.theobromae the cause of fruit rot of grape berries. The other fungicides effective in restricting the growth and sporulation of the fungus were zineb, mancozeb, copper oxychloride, ziride.

5.7.2 Liquid media studies

Benomyl (0.1%), carbendazim (0.1%),captan (0.2%), mancozeb (0.25%) and thiophanate - methyl (0.1%) were found effective against the pathogen and produced zero mg dry mycelial weight of B.theobromae. These results are more or less in agreement with Shinde (1988) who reported that mancozeb and carbendazim completely inhibited the growth of the fungus B.theobromae the cause of die back disease of rose in liquid media.

4.8 Screening of germplasm of mango against die back disease under natural conditions of disease development

It is seen from the results that out of 76 varieties screened one variety namely Bombay butto was observed to

be resistant under natural conditions of disease development. From the rest of the varieties, 58 varieties of mango showed moderately susceptible reaction and 17 varieties showed susceptible reaction. None of the varieties was observed to be highly susceptible under natural conditions of disease development.

Summary and Conclusion

6. SUMMARY

Mango is one of the most important fruit crops in India. During the last 3-4 years the die back disease of mango in Maharashtra in very severe form. Therefore, it was felt necessary to study this disease under different aspects. The studies were carried out on symptomatology of the disease, isolation of pathogen and pathogenecity, morphological, cultural, physiological characters of pathogen, evaluation of fungicides in-vivo and in-vitro and screening of germplasm of mango against this disease under natural conditions of disease development. The results are summarised below :

The symptoms of the disease were characterised as wilting of branches and twigs particularly of the older tree followed by complete defoliation and gave the tree an appearance as if it was schorched by fire. Colouring and darkening of bark at a certain distance from the tip was the external evidence of the disease. Such dark patches are generally seen on young green twigs and were hardly distinguishable in older branches. The upper leaves lost their healthy green colour and gradually turned brown. This was followed by browning of the whole leaf accompanied by the upward rolling of the margin. The brown rolled leaves shrivelled. The shrivelled twig all together was the

characteristics of the advance stage of the disease. Internal browning in the wood tissue was observed on slitting along the long axis.

Isolation of the fungus was carried out from the infected twig. Pathogenecity of the fungus was proved by artificial inoculation by injury method. The pathogenic fungal culture was identified as Botryodiplodia theobromae Pat. and confirmed from the Mycologist, Agarkar Institute, Pune - 411004.

Morphological characters of the pathogen were studied. The fungal pathogen produced cottony white mycelium which was irregularly branched, septate, primarily hyaline but with age turned olive grey to olive black in colour. Pycnidial bodies were formed in six days. Pycniospores were hyaline at early stages of growth and turned brownish. They were single celled primarily and became bicelled at maturity within 19 days of inoculation. They were oval to oblong granular at early stages. The bicelled pycniospores measured 11.16-15.11 X 7.39-11.75 um. In spore germination studies, it was revealed that good spore germination was observed at the end of ten hours after inoculation in host leaf extract followed by one per cent sugar solution.

In the cultural studies, good growth and sporulation were observed on Richard's agar and potato dextrose agar, while good growth and poor sporulation on Sabourd's media.

The minimum, optimum and maximum temperature recorded for growth and sporulation were 15, 25 ± 2 and 30°C, respectively.

The best carbon sources observed was sucrose and glucose followed by mannitol. Good sporulation was observed on maltose, lactose and mannitol and moderate sporulation on glucose was noticed.

The best nitrogen source observed was potassium nitrate followed by peptone and sodium nitrate. Good sporulation on calcium nitrate and sodium nitrate and moderate sporulation on peptone and ammonium nitrate was observed.

Fluoroscent light (850 lux) was the best treatment for abundant septate sporulation of the fungus and good growth followed by room light and sunlight. Black pycnidial bodies were formed on natural host under natural conditions and under artificial conditions.

In in-vivo evaluation of fungicides i.e., in pot culture studies out of seven fungicides evaluated under natural conditions, carbendazim (0.1%) was found to be the most effective in controlling the disease intensity of die back disease of mango after fourth spraying and it has given 80.08 per cent infection control. Next best treatments were thiophanate-methyl (0.1%), captan (0.2%), benomyl (0.1%),

mancozeb (0.25%) showed 72.16, 63.91, 57.40, 55.34 per cent infection control, respectively.

In in-vitro evaluation of fungicides in solid media it was observed that carbendazim (0.1%), thiophanate-methyl (0.1%), captan (0.2%), benomyl (0.1%), mancozeb (0.25%) had completely checked the growth of the fungus. The fungicides aureofungin (0.05%) and copper oxychloride (0.25%) appeared promising in checking the growth of pathogen, in solid media. In liquid media studies carbendazim, thiophanate methyl, captan, benomyl, mancozeb were found 100 per cent effective in checking the growth of pathogen. The fungicides aureofungin was also found promising in production of dry mycelial weight of the pathogen.

In varietal screening programme only one variety namely Bombay butto was observed to be resistant under natural conditions of disease development. Badami, Salem, Kesar, Amrapali, Benarashi Langara proved to be moderately susceptible. Alphonso was more susceptible to this disease. None of the variety was observed as highly susceptible to die back disease of mango.

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Vita

8. VITA

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END