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A study on Black band disease of jute in the Northern part of Bangladesh

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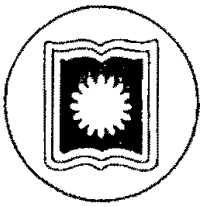
A STUDY ON BLACK BAND DISEASE OF JUTE IN THE NORTHERN PART OF BANGLADESH



Thesis

Submitted to the University of Rajshahi for the Degree of Master of Philosophy in Botany

By

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DEDICATED

TO MY

BELOVED PARENTS

DECLARATION

I do here by declare that the whole of the work now submitted as a thesis entitled "A study on Black band disease of jute in the Northern part of Bangladesh" for the degree of Master of Philosophy in Botany, University of Rajshahi is the result of my own investigation except where due acknowledgement has been given. The thesis has not been concurrently submitted in substance for any other degree.

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CERTIFICATE

This is to certify that the thesis entitled "A study on Black band disease of jute in the Northern part of Bangladesh" is an original work done by Md. Forman Ali for the degree of Master of Philosophy in Botany. The references cited in it have duly been acknowledged. The style and contents of the thesis have been approved and recommended for submission.

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The author

ABSTRACTS

Black band disease of jute (*Corchorus capsularis* L. and *C. olitorius* L.) caused by *Botryodiplodia theobromae* Pat. was studied during June'1999 to October'2001 in Northern part of Bangladesh. The per cent of diseased jute plants were ranged between 19-27%. Optimum conidial germination of *B. theobromae* occurred at 25°-30°C, at pH 6 to 7 and at RH 100%. The fungus prefer 1.5% glucose and 1:15 (w/v) concentrations of cowdung solution for its conidial germination. Whereas, 1% KNO_3 and $NaNO_3$ salt solution gave better germination of conidia. The fungus freely germinates higher (93%) in river water compared to those from other sources of water. The highest mycelial growth and dry weight of *B. theobromae* was observed in PDA and lowest in Richard's medium. On the other hand, formation of conidia showed reverse effect in both the media. When the fungus was cultured on 20% cowdung medium showed better growth. 3% concentration of boron had good effect on mycelial growth of *B. theobromae*. Mustard oil cake is more favorable for the mycelial growth of *B. theobromae* than that of Linseed oil cake. Among the different plant extracts tested, *Datura metel* medium showed better inhibition in culture. The growth of the fungus was found to be affected by the different light conditions and better growth was recorded in continuous light condition. Conidia began to lose their viability after 3 months of storage. Out of seven fungicides tested, Cupravit, Benomyl, Redomil-MZ and Diathane M-45 were most effective fungicides in the inhibition of *B. theobromae* after 5 to 30 minutes immersion. Out of ten plant-extracts tested, two were most effective against *B. theobromae*, three were less effective and five were not effective against *B. theobromae*, when the fungus was immersed for 10 to 30 minutes at 5:1.25 (w/v) concentrations. All the concentration of ginger (*Zingiber officinale*) have inhibitory effects against *B. theobromae*. The growth of *B. theobromae* remained totally unaffected when the cultured medium was exposed to smoke generated from rice straw, tobacco leaf and dhup for 5 to 15 minutes. Growth of *B. theobromae* was completely inhibited on ginger extracts in combination with cowdung extract medium (20% ginger +15% cowdung) after four days of incubation at 28±2°C. There was no inhibitory effect of neem extracts (20% neem +15% cowdung) on this fungus.

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LIST OF ABBREVIATIONS

cm	-	centimeter
CMI	-	Commonwealth Mycological Institute
et al.	-	et alia (= and others)
etc.	-	et cetera (= and the others)
g	-	gram
i.e.	-	id est (= that is)
L	-	Liter
lb	-	pound
LSD	-	List of significance data
mg	-	milligram
ml	-	milliliter
mm	-	millimeter
PA	-	Potato Agar
PCM	-	Paper Chromatography Medium
PDA	-	Potato Dextrose Agar
pH	-	Potential Hydrogen
ppm	-	Parts per million
RH	-	Relative Humidity
sp.	-	Specie
viz.	-	Videlicet (= namely)
w/v	-	Weight per volume
%	-	Percentage
&	-	And
°C	-	Unit of temperature in centigrade scale (Degrees Centigrade)
µ	-	Micron
µm	-	Micrometer
α	-	In measurable

Chapter 1

Introduction

INTRODUCTION

The name Bangladesh and Jute has been closely interlinked throughout the history of its existence as the golden fibre of our country. Jute is the cheapest and the most important of all the textile fibres next to cotton, and is used extensively in the manufacture of different types of packaging materials for various agricultural and industrial products (Anon, 1959). Jute fibre is obtained from the bark of the two cultivated species of the genus *Corchorus,* viz., *C. capsularis* L. and *C. olitorius* L. of the family Tiliaceae. Jute is commonly called *pat* or *nalita* in the central and western regions of Bengal, *pat* or *koshta* in Eastern Bengal, *pata* or *marapata* in Assam, *jhot, jhout,* or *jhuta* in Orissa and *patua* in Bihar. *C. capsularis* is usually called *tita-pat* or *guti-pat* and *C. olitorius Suti, Bogi, Tossa, Deshi or Mitha pat.* In English, jute is known as "Jew's-mallow," which is translated from the old name *Olus judiacum* (Royle, 1855). About 80% of the foreign exchange earnings of Bangladesh depend on Jute (Ekbal, 1989). Jute plays an important role not only in the economy of Bangladesh, but also in the world trade. For such a crop, worldwide market arrangement is very essential for planned market development and industrial uses.

Freshly harvested jute plants contain approximately 75% water, 13% stick, 5% fiber and 7% non-fibrous materials (Ali, 1989). The fibres occur in the phloem tissues of the plants, which are surrounded outside by mostly cortical tissues and inside by xylem tissues. The hard woody core is called jute stick. Before the fibre can be extracted from the woody core, the other embedding tissues containing protein, gums, pectin, sugars, starch and hemicellulose (Ali, 1989).

Jute is a composite fibre containing approximately an intimate mixture of cellulose (58-63%), hemi-cellulose (21-24%), lignin (12-14%). In addition, small quantities of other substances, such as, natural wax (0.4-0.8%), protein (0.8-15%), pectin (0.2-0.5%), traces of tannin and colour pigments are present (Abdullah,

1992a). According to Abdullah (1991), due to the presence of cellulose and lignin jute has some similarity with cotton and wood. The long chain cellulose molecules bonded together with lignin molecules in the jute fibre and also jute sticks, present the possibility of preparing useful compounds from those two compounds. The principal difference between jute and to some extent jute stick with that of wood, can possibly be explained by saying that the cellulose molecules are some what loosely bonded with the lignin molecules in jute, whereas in wood materials the comparatively short chain cellulose molecules usually firmly bonded with lignin material and hence it requires much more drastic chemical treatments to get the cellulose from wood than from jute. Actually jute is the purest from of natural cellulose, which usually contains a very small amount of lignin and other impurities.

Jute plays a very important role in the economy of Bangladesh. Our agricultural community is dependant to a large extent on jute and jute product. This sturdy fibre has so far mainly been used in the production of hessian, carpet backing and gunny bags. Another important use is for linoleum backing and jute yarns are largely used, in conjunction with cotton yarns, for carpets. It is considered to be an important fibre crop and regarded as the foremost bast fibre and second most important textile fibre after cotton (Kundu, 1956; Kirby, 1963). Jute stick cellulose can be successfully used in this type of industry for making good quality paper and rayon. The jute stick thus procured can be put into a variety of uses, this includes (i) manufacture of particle board (ii) pressed sheet boards (iii) paper and rayon from purified cellulose (iv) a variety of chemicals manufactured either in sits when producing cellulose pulp or separately, from the lignin residue of jute stick. Cellulose is mostly present in crystalline part whereas, non-crystalline amorphous part consists of lignin and hemi-cellulose. So, this complex and peculiar three-dimensional picture of jute predominantly reflects physiochemical properties of jute fibre.

Jute grows extensively in almost all part of Bangladesh except the hilly and coastal areas. The temperature, wet-warm, humid climate, well drained loamy soil, long day light of Bangladesh and part of India, all are very suitable for the rapid growth of jute. So, ecologically, jute is the inhabitant of this ecosystem. The people of Bangladesh and this part of the world were accustomed with the uses of jute in different household activities from farmyard to health care. There is a great demand for this fibre in the western countries. So jute is an important foreign exchange earning commercial crop of Bangladesh.

Jute is an annual plant, which is slender without branching, leaves grow directly on the stem. In Bangladesh, Jute is grouped into two local categories as *Deshi* and *Tossa* jute on the basis of morphological and physiological characteristics. Like other agricultural crops jute is also subjected to many diseases. Both the cultivated species of jute *Corchorus capsularis* (White or Deshi) and *C. olitorius* (Tossa or Bogi) suffer from various diseases. Beginning with the seedling stage, the jute crop is liable to be attacked by various diseases, mostly caused by fungi. Seeds, roots and all parts of the plant-body are subject to attack, and the yield as well as the quality are affected. The fungi causing the important disease of jute are *Macrophomina phaseoli* (Maubl) Ashby, *Sclerotium rolfsi* Sacc., *Diplodia corchori* Syd. and *Colletotrichum corchori* Ikata & Tanaka. Besides, a number of other fungi and bacteria attack the plants at various stages of growth and cause damage. Ghose and Basak (1951) have recently reported that chlorosis may be caused by a virus. A number of grafts were made between chlortotic and non-chlortotic plants and *vice-versa*, using in-arch and cleft methods of grafting. It was found that chlorosis was transmitted in both the directions, that is, the chlortotic stock transmitted chlorosis to the healthy scion. It was, however, noticed that transmission was slightly delayed in the case of the latter.

Although an appreciable amount of work has been done on the general aspect of different jute diseases and methods of controlling them, but literature on the black band disease is scanty. Scientists of Bangladesh Jute Research Institute have done extensive research on different diseases. Biswas *et al.* (1976) have done disease reaction of wild species of *Corchorus* against different races of *Macrophomina phaseolina* (Tassi) Gold as, under greenhouse conditions. Ahmed and Ahmed (1977) investigated some new races of *Macrophomina phaseolina* (Tassi) Gold from jute seeds diseased plants of *Corchorus* spp. Bhadra and Ahmed (1978) studied the effect of treatments on fungal population of a jute field soil. Sultana and Jalil (1979) also evaluate some pesticides for control of jute white mite, *Polyphagotarsonium latus* (Banks). Effect of different weed control practices on weed growth and performance of jute has been performed by Ahmed, 1979. Bhadra and Ahmed (1979) studied survival of *Macrophomina phaseolina* (Tassi) Gold, causing stem- rot disease of jute in different soil conditions. Biswas *et al.* (1980) have done field resistance of jute cultivars against stem rot disease (*Macrophomina phaseolina*). Banu *et al.* (1998-99) have screened of jute, *Corchorus* spp. for resistance to yellow mite, *Polyphagotarsonium latus* (Banks).

Black band disease is caused by *Botyodiplodia theobromae* Pat. Shaw (1921) described the disease 'black band' caused by *Diplodia corchori* Syd. The fungus is mainly a wound parasite and a very weak one. The disease is usually prevalent during the middle of July. Attacked plants wilt and are completely defoliated. Pycnidia develop all over the stem which appear black due to the development of sooty spore masses in abundance. Epidemics were once reported from Rangpur and Kishoreganj (E. Pakistan). After 1948, "black band" as a major disease has not been reported. Now a days, the disease is spreading severely from place to place in the northern areas of Bangladesh and cause heavy losses in jute cultivation. The fungus develops a dense black band around the stem at about 2-3 feet above the ground level. Such affected plants ultimately loose all leaves and remain as dry black stems. The bark of the main stem splits longitudinally at the

later stages of the disease development. The fibres turn brown and dry, the pathogen produces minute special black pycnidia on the blackened stem. Superficially these pycnidia are confused with those of the stem not. However, on rubbing hands up and down a stem, the fingers become black due to blackish pycnidiospores formed inside the pycnidia. Black band is a relatively serious problem for jute growers, the economic importance of the disease has increased in jute fibre trade in foreign countries.

Behaviour of a fungus/pathogen depends upon its nutritional response, phytopathogenic organisms express a similarity in broader behavior for their basic nutritional needs, yet they maintain their individuality for the choice of specific substances (Lilly and Barnett, 1951). Micro-organisms possess a remarkable capacity to utilize a wide range of organic and inorganic substances. Now a days, most of the organisms are grown *in vitro* by supplying proper nutrients.

It is now well established that phytopathogens show greater diversities in their ability to utilize the same elements from different nutrient media. This selective property of pathogen has resulted in the formulation of many natural, semi-synthetic and synthetic culture media. These culture media always contain essential element needed for the proper growth and sporulation of the organism / pathogens. Moreover, the suitability of a particular medium varies with fungi. Though, "media differ with respect to constituents and concentrations" (Lilly and Barnett, 1951), the selection of basal medium becomes indispensable for the physiological studies of fungi.

The growth of fungi is profoundly affected by environment. Temperature is one of the important environmental factors, which influences the growth and development of fungi. Some fungi multiply severely at low temperature, others at high temperature. So fungi/pathogen differ in their optimum temperature for growth, sporulation and infection (Bruehl and Cunfer, 1971).

Fungi exhibit varying responses to light depending on the light intensity, light quality, duration of exposure and temperature. The effect of light is

dependent on temperature. Exposure to light is needed by some fungi for sporulation (Marsh *et al.*, 1959), whereas other fungi sporulate better in darkness (Shoemaker, 1955).

Humidity is another important environmental factor, which influences disease development and conidial germination of fungi and occur over a wide range of relative humidities (Jacome *et al.*, 1991).

Hydrogen-ion concentration plays a major role in the adaptability of organisms to their nutrient environment. Generally the micro-organisms are known to tolerate a wide range of pH, which may differ from species to species.

On the basis of this views expressed above the present investigation has been under taken with an aim to find out the causal organism involved in the black band disease of jute in the Northern part of Bangladesh and build up an economically and environmentally sustainable control measure of this disease with the help of some chemical compound and plant based extracts.

In course of the present investigation, keeping all the above in view, comprehensive studies were undertaken with the following objectives:

1. Survey of the Black band disease of jute in Northern areas of Bangladesh.
2. Isolation, purification, characterization and identification of the causal organism.
3. Factors affecting development of the fungus, viz. effect of temperature, hydrogen-ion concentrations and humidities, on the germination of conidia.
4. Nutritional and environmental factors affecting growth, sporulation and germination on the conidia of the test pathogen.
5. Efficacy of some chemical fungicides were tested against the causal organism.
6. Effect of different plant extracts and smokes on the inhibition of fungal growth and germination.

Chapter 2

Review of Literature

REVIEW OF LITERATURE

Corchorus capsularis L. and *Corchorus olitorius* L. the two cultivated species of jute are susceptible to various fungal diseases. In Bangladesh jute cultivation faces several serious fungal diseases. Black band disease is a serious fungal disease caused by *Botryodiplodia theobromae* Pat. Twenty-eight isolates of *B. theobromae* were isolated from diseased seeds and stems of Jute in Bangladesh by Haque *et al.* (1973).

At first Shaw (1912) reported the stem rot disease of jute from India. No other single disease of jute causes more damage than the stem rot due to *Macrophomina phaseoli* (Maubl, Ashby).

In 1921 Shaw found the presence of another organism producing pycnidia on the jute plants. The mature spores, which are brown and one septate. The species was known as *Diplodia corchori.* Later this was universally known as *Botryodiplodia theobromae* (Wadud and Ahmed, 1962).

Literature on this score is quite scanty, so the review work has been done mostly on related work with other diseases and other fungi on survey of diseases on nutrition and environmental factors affecting growth, sporulation, germination, chemical control measures and plant extract.

2.1 GERMINATION OF SPORES:

The process of spore germination and the factors influencing it have captured attention of scientific workers from the nineteenth century. Since the publication of Hoffman's (1860) detailed investigations on spore germination, much work has been done in this field and some important reviews have been published.

The factors influencing spore germination may be internal or external. The age of the spore is important internal factor and the external requirements for

spore germination are water, humidity, nutrients, hydrogen ion concentration (pH values) and temperature.

Pure water is sufficient for the germination of many fungi but some require an additional supply of nutrients or other substances for successful germination.

Experimental conducted by Clayton (1942) indicated that even at high humidities, spores of fungi could not germinate and they required free water, while other were adapted to use water vapor.

Hutchinson (1929) reported that 28°C as the optimum temperature for germination of spore of *Botryodiplodia theobromae.* He observed germtube production from one or both end of the spore.

Lin (1945) while working on *Glomerella cingulata* found that for successful germination the fungus required carbohydrates, nitrogen, phosphorus and magnesium. Temperature has marked influence on spore germination. The minimum, optimum and maximum temperature for spore germination of different fungi shows greater variations. Usually the minimum lies between 0°C and 10°C, optimum 20°C and 30°C and maximum 35°C and 40°C, but the exception are not in infrequent.

Singh and Singh (1951) reported that the minimum optimum and maximum temperatures for germination of spore of *Ascochyta sorghi* was 25°C, 30-32°C and above 38°C respectively.

Patel (1952) noticed that the minimum, optimum and maximum temperature for germination of *Colletotrichum* sp. were 10°C, 26-28°C and 41°C respectively.

According to Walker (1969) the conidia of *Alternaria solani* germinate at the optimum temperature of 28°C-30°C within 35 to 45 minutes.

Bhargava (1971) studies the effect of various environmental factors of the spore germination of *Fusarium solani* and *Botryodiplodia ananassae*. He observed

the spore germination of the two fungi was best between 25°C & 30°C and it decreased with any decrease or increase of temperature. A temperature of 8°C inhibited the spore germination of both the fungi. At 12°C, only 54 per cent spores of *F. solani* germinated after an incubation of 22 hours, while spores of *B. ananassae* failed to germinate. At higher temperature of 40°C, 81 per cent spores of *B. ananassae* germinated often an incubation of 22 hours while, the spores of *F. solani* completely failed to germinate.

Bedi and singh (1972) observed that growth of *Alternaria alternata* took place between 5-40°C with the optimum at 30°C. Below 10°C and above 35°C the growth was very slow. The growth was completely suppressed at temperature bellow 5°C and above 40°C. The pathogen sporulated at a temperature range of 10°C-30°C with maximum sporulation at 25°C.

Quimio and Quimio (1975) reported that *Colletotrichum gloeosporioides* grew at 15°C-35°C (optimum 25°-35°C). He found that at 10°C growth was completely inhibited. The rate of decay of inoculated mango fruits followed the same trend as the growth of the fungus at different temp. Both *in vivo* and *in vitro* low temp. was not lethal since growth of the fungus and fruit lesion development were resumed when cultures and fruits were at ambient temps (30±2°C). Low temp. (10°C) can more or less prevent the disease since the rate of decay was slower or previously chilled fruits than on unchilled ones.

Yadav and Agnihotri (1980) observed within 6 hours at 25°C on the moist surface. They observed that there was no difference in conidial germination of *Pyricularia penniseti* at 22°, 25° and 27°C in 12, 18 and 24 hours, but the difference was marked at 6 hours. Spore germinated better at 25°C and the rate decreased rapidly above 27°C.

During the study of *Altenaria porri* Khare and Neema (1982) observed that cent percent spore germination occurred *in vitro* within four hours at 22°C, while

maximum germination was recorded within six hours at 25°C on the host surface. They also recorded relative humidity of 100% for more than four hours at 22°-25°C.

Ahmed (1985) reported that *Colletotrichum gloeosporiodes* Penz. the causal organism of "Anthraenose" or "Rot" of many important fruits of tropical and subtropical countries, grew and sporulated at 15°-35°C, the optimum being 25°-30°C, both on PDA and its host fruit. There was no growth of the organism at 10°C, in either case, light is not necessary for the growth but it enhanced the sporulation.

Kore and Kharwade (1987) observed that 50 % germination occurred in Tinda fruit juice followed by 2% sucrose solution (32 %) respectively. They also found that, of different media like, Tinda fruit juice, 2% sucrose solution, sterile water, tap and distilled water are sufficient for conidial germination.

Chandra Mohanan *et al.* (1987) studied that the spore of *Colletotrichum gloeosporioides* germinated maximum in tap water.

Singh and Chauhan (1988) observed the effect of temperature on germination of zoospores of *Phytophthora drechsleri* f.sp. *Cajani.* They found that maximum temperature for germination was 30°C and minimum was 10°C.

Rewal and Grewal (1989) reported that minimum, optimum and maximum temperatures for the germination of conidia of all the three strains of *Botrytis cinerea* were found to be 5°, 20° and 30°C. Mycelial strain B_4 however more than 50% germination at 10°-30°C sclerotial strain B_5 at15°-25°C and sporulation strain B_1 at 20°-25°C.

Jacome *et al.* (1991) observed that conidia of *Mycosphaerella fijiensis* var. *difformis* germinate maximum in free water. Pure water is sufficient for the germination of spores of many fungi, but some require an additional supply of nutrients or other substances for successful germination.

Meah (1993) studied the effect of temperature on disease development of *Botryodiplodia theobromae* Pat. on mango and found the highest rate at 32°C and the lowest in 13°C. No disease was observed at 10°C until 32 days after inoculation.

Appaji and Thakur (1994) reported *in vitro* experimental result of pearl millet downey mildew by *Sclerospora graminicola* that sugar did not induce germination of sporangium. Glucose was found to show least inhibitory effect.

Effect of temperature on conidial germination of chilli fruit rot pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed conidia germinated over the temperatures range of 10-35°C, but the optimum range was 20-25°C.

Alam *et al.* (2002) reported that conidial germination of *Bipolaris sorokiniana* was the highest (100%) in 1.5% and the lowest (5%) in 3.5% KNO_3 solutions within 48 and 2 hours of incubation period respectively. In $NaNO_3$ the highest (83%) conidial germination was recorded in 1% and the lowest (3%) in 3.5% solution within 48 and 2 hours of incubation period. With the increase or decrease of KNO_3 in solution the rate of germination was increased or decreased. But in $NaNO_3$ solution, conidial germination was gradually decreased with the increase of $NaNO_3$ in solution.

2.2 EFFECT OF RH:

Vicente (1983) studied the effect of relative humidity (RH) on the germination of ascospores of *Mycosphaerella muscicola,* causal agent of sigatoka disease of banana. He found that germination of ascospore takes place at RH 88% and release of ascospore from the mature perithecia began 20 minutes after leaf tissues became wet, reaching a maximum at 30-40 minutes and ending after 60-80 minutes.

Lim and Tang (1985) observed the effect of RH on growth and sporulation of *C. gloeosporiodes* on oatmeal agar and found that setae are most abundant at 80-90% RH.

Chandra Mohanan *et al.* (1987) stated the effect of RH of the infection by *C. gloeosporiodes* taken from five days old cultures grown on oatmeal agar. They showed that infection increased linearly with the increase in time of exposure to high RH and exposure to high humidity for 27 hours is ideal.

Kore and Kharwade (1987) studied the effect of RH on the growth and sporulation of *F. oxysporum* causing fruit rot of round gourd on PDA. They observed that RH of 70-90% shows good growth of the fungus whereas, growth is retarded at 100% RH. They also observed that 30 and 50% RH gives poor growth of the fungus.

Rewal and Grewal (1989) observed the effect of RH on the germination of conidia of three strains of *Botrytis cinerea* infection chickpea. They found strain B_1 needs 93-100%, strain B_4 81-100% and B_5 72-100% RH for their conidial germination.

Jacome *et al.* (1991) reported the effect of RH on germination of conidia of three isolated and ascospore of one isolate of *Mycosphaerella fijiensis* var. *difformis*. They showed that maximum germination is in free water, germination decreases at lower RH. Conidia germinate over a wider range of RH at 92-100% that of ascospores at 98-100%. Ascospores germinate earlier than conidia.

Effect of RH on conidial germination of chilli fruit rot pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed that conidial germination was initiated in 2 - 6 hours at 90 and 75 % relative humidity.

2.3 EFFECT OF pH:

Hydrogen ion concentration of the medium has a profound influence of overall metabolic activities in fungi. Highly acidic or alkaline media usually inhibit the growth of fungi and the minimum, optimum and maximum pH values as well as the pH range for growth and sporulation very considerably. Most of the fungi cease to grow below pH 2 and pH 9 (Wolf and Wolf, 1947). There are however some exception e.g. Meacham (1918) reported that *Merullins lacrymans* could grow at pH 10, whereas *Fusarium bullatum* and *Penicillin variable* has been reported to grow even at pH 11 (Johnson, 1923). Smart (1937) reported that spores of *Fuligo septica* germinated from pH 2.0-10.0.

Hawker (1950) stated that most fungi grew best at neutral reactions, that is, at pH 7 or slightly on the acid side of the neutral. He reported that the growth of *Fusarium fractigenum* on Richard's solution of pH-4.6 gradually raised the pH until the reaction turned strongly alkaline. This shifting of pH is a frequent phenomenon. Cochrana (1958) reviewed that pH optima of a number of plant pathogenic fungi and showed that most of them grew best between an initial pH of 5.0 to 6.5.

Lilly and Barnett (1951) stated that fungi as a result of their pH metabolic activities, ordinarily changed the pH of the media in which they grew. Since fungi differed in their metabolic activity and rate of growth, the pH changes produced in the cultural medium differed. The patterns of pH changes for same fungus depended upon the composition and concentration of the media used.

Booth (1971) reported that the pH of the culture media for *Fusarium* sp. is maintained at 6.5 in CMI.

Satya Vir and Grewal (1972) found that *F. caeruleum grew* at different pH levels ranging from 2.0 to 8.0. No growth was obtained at 2.0 Optimum growth of the fungus was recorded at pH 6.0.

Mathur and Sarbhoy (1977) recorded the maximum growth and excellent sporulation of *S. rolfsii* and *A. alternata* at pH 5.5.

Kumar and Singh (1987) studied that soaking of teliospores of *Neovossia horrida* in water for three days at pH 5.00 and showed significant increased of germination of spores.

Du *et al.* (1988) stated that maximum growth of the fungus *M. rofidium* was at range of pH 5.4-8.5 and suitable range at pH 4.0-9.5, when cultured on PDA.

Siddique and Mridha (1989) reported the effect of pH on growth and sporulation of *C. capsici,* a common pathogenic fungus of chilli (*Capsicum* sp.). They found that best growth and abundant sporulation are around at pH 5 in PDA medium.

Chauhan and Singh (1991) reported the effect of pH of the germination of zoospores of *Phytophthora drechsleria* f.sp. *conjani.* They found that the Zoospores germination in a wide rage of pH at 0.4-11.0. The maximum germination at 73% was observed at pH 7.5.

2.4 NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING THE GROWTH AND SPORULATION:

2.4.1. Effect of Media:

For the cultivation of fungi in the laboratory it is necessary to supply in the artificial medium those essential and compound they require for the synthesis of their food and for the operation of their life processes.

Brefeld (1881) was one of the pioneers to grow fungi in pure culture. For the cultivations of fungi in the laboratory, it is necessary to furnish to the medium those essential elements and compounds they require for the synthesis of their all constituents and for the operation of their life processes. Earlier studies dealing with nutrition of fungi revolved diversities in their ability to utilized the same

element from different nutrient media. The selective property of fungi has resulted is formulating a good number of synthetic, semisynthetic and natural media.

Gordon (1952) in winnipeg found potato-sucrose-agar eminently suitable for *Fusarium* culture and this is the standard medium used at the CMI.

Toussoun *et al.* (1960) demonstrated in case of *F. solani* f. sp. *phaseoli* on enhanced nitrogen while inoculum was growth on organic nitrogen and while inoculum growth on the media containing glucose delayed pathogenesis. It also indicates that there was direct relationship between the colony growth and sporulation of the fungus in PDA.

Armstrong and Armstrong (1965) recorded maximum growth of *Fusarium* sp. on Armstrong-Fusarium media.

Cappellini and Peterson (1965) found that they could stimulated conidial formation in *F. agraminearum* by using Carboxy-methyl-cellulose. They macerated a plate culture in 100 ml of sterile distilled water for 30 seconds in a warming blender and 1 ml of this suspension was added to each test-flask. The flasks were then shaken on a rotatory shaker at approximately 2500 rpm and abundant macro-conidia were present after four days.

Konger (1971) observed that almost all the media tried. Potato-dextrose-agar was found to be most suitable for growth of mycellium in *F. axysporum* and *F. radicicola.*

Booth (1971) reported that the pH of the culture media for *Fusarium* sp. is maintained at 6.5 in CMI.

Singh and Gupta (1972) observed that *F. moniliforme* grew and sporulated well on potato-dextrose agar medium.

Jhamaria (1972) reported that different media showed marked variability in growth, sporulation and colony characters of *F. oxysporum:* PDA, Richard's and Czapek's-agar media provided maximum growth and sporulation of the fungus.

Ghosh and Sen (1973) reported that nitrogen requirements of four isolates of *Macrophomina phaseoli* (Maubl.) Ashby. They found that all 4 isolates of *M. phaseoli* (*M. phaseolina*) from jute utilized ammonium, nitrate, nitrite and organic N. Some amino acids were better N sources than inorganic ones. A wide range of N Conc. was utilized when there was sufficient C present. N was not the important factor in increasing growth when the C/N ration was changed.

Tewari and Dath (1984) studied the effect of leaf extract media of some plants on the growth of three fungal pathogens of rice. None of media tested favored both growth and sporulation of *Pyricularia oryzae* and *Corticium sasakii.* Rice leaf extract medium favored the growth as well as sporulation of *Drechslera oryzae.*

Chandra Mohanan *et al.* (1987) reported that incase of *C. gloeosporioides*, Oat-meal-agar was the best medium for germination and degree of infection. Conidial germination was maximum in tap water and also the degree of infection.

El-Abyad and Afifi (1989) observed that growth and sporulation of *F. oxysporum* f.sp. betae in its saprophytic phase, were likely to reach opt. within the temperature range 25°-30°C. Sporulation was maximum at 100% RH. Growth was highly enhanced in acidic sandy-clay soils (50:50, w/w) adjusted to 40-60% of its moisture holding capacity. Acidic reactions were favourable for the population of macroconidia. A sucrose concentration of 3% stimulated growth and sporulation. Although growth was favoured by the presence of 0.8% concentration of organic nitrogen source (asparagine), sporulation was favoured by the presence of 1% $NaNO_3$. A balanced high C: N ratio (1.5:1.5) stimulated both growth and sporulation of the fungus.

Siddique and Mridha (1989) studied the effect of media on growth and sporulation of *C. capsici,* a common pathogenic fungus of chilli (*Capsicum* sp.). They found that among the culture media PDA, tomato-sucrose-agar, malt-estract-

agar, glucose-peptone-agar, coon's agar and host extract-agar produce abundant sporulation and moderate colony growth is obtained in malt-extract-agar medium. They also found that both colony growth and sporulation are fairly well in host extract-agar medium and poor in glucose-peptone-agar medium. Best growth and abundant sporulation are found in PDA medium.

Quroshi and Meah (1991) reported the effect of different medium for the growth and sporulation of fungus *Botryodiplodia theobromae.* They found that growth of the fungus in faster on Richard's agar medium PDA and poorest on Czapeck's agar medium. Highest number of pycnidia is obtained on mango leaf extract agar followed by PDA medium.

Chowdhury *et al.* (1992) studied the effect of growth and sclerotia productions *in vitro* using a soil plate method of the fungus *Sclerotium rolfsii* on different textural soil and its response to different chemicals. They found that maximum mycelial growth at 8.5 cm and sclerotia formation on sandy loam (448.33) and silty clay soil (373.33), where range of pH from 6.1-6.5 respectively.

Rahman *et al.* (1993) reported that effect of nitrogen and carbon sources on growth of *Fusarium oxysporum* and *Sclerotium rolfsii* and observed details are given of the effects of 5 inorganic and 10 organic N sources and of 12 C sources on *F. oxysporum* and *S. rolfsii.*

Hebbar *et al.* (1996) observed that the mycelial growth and conidia and chlamydospore formation were different for the 3 strains and varied with the substrate used. The quantity of conidia and chlamydospores produced depended on the concentration of the substrates. Irrespective of carbon: nitrogen ratios, chlamydospores were formed rapidly in liquid media and in greater amount on substrates with a low utilizable carbon content (aqueous extracts of soyabean hull fibre and corn cob) than on those with higher utilizable carbon content (potato dextrose broth, aqueous extracts of cotton seeds and molasses yeast extract broth).

In liquid cultures, increases in the concentration of substrates high in utilizable carbon resulted in reduced chlamydospore formation, however, this was far less in those with a lower carbon content.

The fungi-toxicity or antifungal activity of leaf extracts of *Azadirachta indica, Chromolaena odorata, Lantana camara, Piper colubrinum* and *Strychnos nuxvomica* have been reported by Anandarj and Leela (1996) on the different growth phases of *Phytophthora capsici.* They found that mycelial growth, sprongial production, zoospore production and release, and zoospore germination were completing by *C. odorata* extracts at 2% concentration. *A. indica* extracts also acted similarly but mycelial growth was inhibited only by 75.5%. *P. colubrinum* extracts inhibited mycelial growth and sporangial production whereas, sporangial germination and zoospore germination were inhibited by 23.71 and 20.13 % even at 2 % concentration. The extracts of *S. nuxvomica* had inhibitory effect on sporangial production at 0.25 % concentration whereas, on the other phases of the fungus, it was not very effective. The extracts from *L. camara* were effective.

Srivastava and Lal (1997) observed an *in vitro* test indicated fungicidal properties in aqueous leaf extracts of *Calotropis procera, Azadirachta indica, Lantana camara* and *Ocimum basilicum* aginst *Curvularia tuberculata* and *Alternaria alternata.*

Sindhan *et al.* (1999) reported the effect of some plant extracts on the vegetative growth of root rot causing fungi *Rhizoctonia solani* and *Rhizoctonia bataticola.* They found that extracts of all the plants were toxic to the mycelial gowth of *R. solani* and *R. balaticola* and bulb and rhizomes extracts of *A. cepa, A. sativum, Z. officinales* and leaf extracts of *A. indica* were more toxic to both the fungi as compared to other extracts.

Basak and Paul (1999) has observed that plant extracts of *Azadirachta indica*, *Polygonium hydropiper*, *Lantana camara*, *Cassia tora* and *Moringa oleifera* had suppressive effect on mycelial growth of six major fruit rot fungal pathogens of chilli.

Basak *et al.* (2002) studies on comparative efficacy and *in vitro* activity of cow urine and cow dung for controlling root rot disease of cucumber caused by *Fusarium solani* f. sp. *cucarbitae* Snyder & Hansen following slide germination and mycelial growth inhibition tests. They observed that both germination of conidia and the percentage inhibition of mycelial growth decreased or suppressed and varied greatly with respect to different hour and days of incubation and kind of bio-matters. In between two bio-matters cow urine was found more effective than that of cow dung in conidial germination. In this test cow dung potato dextrose agar (CDPDA) had less efficacy in suppression of the percentage inhibition of mycelial growth.

2.4.2 Effect of Light:

Booth (1971) observed that diffused light favored spore germination in *Fusarium* species.

Tiwari and Yadav (1977) reported that vegetative growth was more in violet and green light while sporulation was more in red and yellow light in two strains of *Choanephora cucurbitarum*. Vegetative growth and sporulation were minimum in continuous dark and continuous artificial light.

Ahmed (1985) studied the effects of light on the growth and sporulation of *Colletotrichum gloeosporioides*, the causal organism of anthracnose or rot of many important fruits of tropical and sub-tropical countries. He observed that light was not necessary for the growth but enhanced the sporulation of *C. gloeosporioides*.

Rewal and Grewal (1989) reported conidia germination of *Botrytis cinerea*, strain B1 in continuous light, strain B4 in alternating 12 hours of light/darkness and strain B5 in complete darkness.

Chauhan and Singh (1991) found that light had also marked inhibitory effect on zoospore germination of *Phytophthora drechsleri* f. sp. *cajani*. Maximum germination (84.7%) was obtained in complete darkness. The reduction in germination ensured following exposure of zoospore to light of low intensity but at 500 lux the germination was completely inhibited. The observation recorded in the present work resembles the work done earlier and showed light had marked variations of germination of spores of *F. oxysporum* f. sp. *vasinfectum*.

Effect of various culture conditions on *A. alternata* and *F. oxysporum*: 1. culture media, temperature, age and carbon source have been reported by Osman *et al.* (1992). They found that optimum conditions were achieved by incubating cultures for 8 days at 30°C using sucrose as the carbon source.

Effect of light on mycelial growth of chilli fruit rot pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed that mycelial growth of *A. tenuis* was the highest in continuous light and the lowest in complete darkness.

Alam *et al.* (2001) reported the effect of temperature, light and media on growth, sporulation, formation of pigments and picnidia of *Botroydiplodia theobromae* Pat. They found that light was not necessary for growth, but it enhance the sporulation of this fungus.

2.4.3. Viability of Conidia:

In nature, conidia of pathogenic fungi are subjected to a variety of adverse condition, which may result in death of the conidia before then can successfully attack a susceptible host.

Dastur (1916) reported that conidia in clumps survived desiccation for as long as nine months, whereas drying quickly killed thin films of spores. Similar result observed Simmond and Mitchell (1940).

Nishikado *et al.* (1937) reported that the spore of *Helminthosporium oryzae* survived for 34 months at 0°, 5°, 15° and 20°C and for 28-29 months at 30°C but lost its viability when stored for more than five months at 34°C.

Wolf and Wolf (1947) reported that fungal spore retained their viability at higher temperatures when subjected to dry heat to moist heat.

Bega and Smith (1962) made a study of time temperature relationship on thermal inactivation of sclerotia of *M. phaseoli* in hot water. They observed that the sclerotia survived 50°C for 60 minutes, but were inactivated in 15minutes at 55°C.

Verma (1972) reported that spores of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were stored in sterilized polythene bags for various periods at room temperature and were germinated to study the viability. The spores of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were viable up to 11 months. Spore germination was at best 25° C in case of *Colletotrichum gloeosporioides* and at 30°C in case of *Botryodiplodia theobromae*.

2.5 CONTROL MEASURES:

In the recent past, considerable work has been done in the field of disease control through chemicals, which are called fungicides. The fungicides act on or interrupt the metabolic system of a pathogen to control the disease caused by the pathogens. The fungicides are basically toxic to all pathogens. In selecting a suitable fungicide should be highly toxic to the pathogen and secondly, the sensitivity of the host to the fungicide should be relatively low.

Lilly and Barnett (1951) reported that there was no useful universal fungicide, because different species of fungi exhibit greater variation in their sensitivity to various fungicides. A fungicide, which in lethal or highly toxic to a particular fungus, may be totally ineffective against another fungus.

Aragaki and Ishii (1960) reported that the use of chemicals has been the only feasible means of controlling mango anthracnose in all its manifestations. Using Haden and Wootten as test varieties, spray tests were conducted during 1955 and 1957. Increased Haden yield was obtained with zineb and good fruit protection was obtain with captan in the 1955 test. Increased yields and a high degree of fruit protection were obtained with all the treatments of the 1957 test.

Narain and Panigrahi (1971) observed the efficacy of some antifungal compounds on the conidial germination of *Colletotrichum capsici* and percentage of infection incited C. *capsici* on the ripe fruit of G-2 chilli variety in field when treated with 8 antifungal compounds. There results indicate that Ziram was the most effective compound to restrict the conidial germination of C. *capsici,* even at 500ppm. Again Ziram was found to be most effective for controlling the infection by C. *capsici* on the test plants under field conditions.

Howare (1972) observed that out of five fungicides evaluated *in vitro* against *Fusarium lini,* benomyl, chloronels, vitavax and pantavax inhibited the growth of the fungus where as aureofungin failed to inhibit the growth.

Khanna and Chandra (1975) studied *in vitro* and found that benomyl and aretan were highly toxic to the growth of *F. moniliformae* and *F. roseum.* Aureofungin is found effective only at higher concentration. The fruit treated with benomyl retained their edible quality.

Kolte (1976) has found the systematic fungicides benlate, bavistin, brestanol and cercobin highly effective against *Cercospora* leaf of groundnut.

Mendoza (1977) proposed that fungicide applications (Daconil 75wp [chlorothalonil], dithane M-45 [mancozeb] or manzate 80 (maneb) at 2.64 g/l after bud break, at fruit set and 10 days before harvest gave good control of *Colletotrichum gloeosporioides* on mango. He found that climate the need for post-harvest treatments.

Teo and Kuch (1982, 1984) reported that in lab, tests several fungicides were promising against mycelial growth of *Colletotrichum gloeosporioides,* the cause of anthracnose. They found that velvet blight (*Septobasidium bogoriense*) was prevented from recurring on twigs from which the mycelium was scraped prior to painting with a paste of copper oxychloride, chlorothalonil or stabilised Bordeaus.

Kaul (1984) observed that of the six systemic fungicides tested, almost all the systemie fungicides were in effective against *Rhizopus stolonifer.* Thionbendazole (TBZ) at both 1000 and 500-ppm concentration were superior followed by 1000-ppm carbendazim. No significant difference was observed in the efficiency of benomyl and theophanatemethyl both at 1000 and 500 ppm was effective. Result of benomyl, carbendazim and thiophanatemethyl at lower concentration were not encouraging either.

Rosenberger and Mayer (1985) reported that combinations of diphenylamine (DPA) at 1000-2000 µg/ml and benomyl, thiabenazole or thiophantamate methyl provided better control of benomyl resistant *Penicillium expensum* in incubated apples stored at 2.2-4.4°C either DPA or the fungicides

used alone. The addition of DPA did not significantly affect control, achieved with the fungicides when fruits were stored at 16-22°C. Comparisons of mycelial growth rates for three binomyl resistant and four benomyl sensitive isolates in the presence of varying concentration of bynomyl, DPA or benomyl plus DPA showed benomyl resistant isolates were more sensitive to DPA than benomyl sensitive isolates.

Lim and Wai (1986) stated that benomyl propiconazol, captafol, captan, chlorathatonil and mancozeb, tested against *C. gloeosporioids.* They found that captafol is the most fungistatic, inhibiting mycelial growth and sporulation at ED_{50} µm/ml respectively and suppressing germination, germ tube growth and appressoria formation at ED_{50}<0.01 µm/ml.

Mehta *et al.* (1986) reported that potassium metabisulphate at 600 ppm, ascorbic acid at 1100 ppm, naphthalene acetic acid at 200 ppm and bavistin at 2000 ppm spray done 15 years old Dashehari and Chumsa mangoes effectively controlled malformation. Bavistin proved most effective with 95 to 91.3% disease reductions respectively.

Natarajan and Lalithakumari (1987) reported that the antifungal activity of the leaf extract of *Lawsonia inermis* on *D. oryzae* was tested at 1:40 dilution (Ec 50 concentration) by measuring the growth. The antifungal factor contained in leaf identified as 2-dhydroxy-1, 4 naphthoquinone (Lawsone). Under *in vivo* condition, foliar spray of the leaf extract effectively controlled disease than the seed treatment.

Tewari *et al.* (1988) evaluated minimum inhibitory concentration of 5 fungicides as bavistin, benlate, dithane M-45, tecto-40 and calixin using the poisoned food technique *in vitro* and all except dithaneM-45 was also tested *in vivo*. Bavistin and tecto-40 were most effective *in vitro* and gave 95 and 85% control, respectively. Dithane M-45 was not effective. Benlate and calixin gave 70 and 80% control, respectively.

Sekhar *et al.* (1989) reported that during 1987-88 and 1988-89, field trails are carried out on *Z. mauritiana* cv. kaithali infected by *Prarthgada zizyphi* of 6 fungicides applied as sprays, bavistin to at 0.1% gave the lowest disease incidence followed by at 0.2% dithane M-45.

Singh and Agarwal (1989) observed that carbendazim (0.1%) and propionic acid (1.0%) reduced the infection of both *F. moniliformae* and *Phoma sorghina* significantly in seeds. The use of captafol (0.2%) and triademefon proved better for control of *F. moniliformae*.

Siddaramaiah and Hegde (1990) reported that out of ten fungicides tested in *in vitro,* bavistin completely inhibited the growth of *Cercospora moricola* at 1000 ppm. Next best was benomyl followed by dithane M-45, aureofungin sol, dithane Z-78, and decolin. Under field condition, out of ten fungicides tested, bavistin (0.05%) was found to be highly effective in checking *Cercospora* leaf spot of mulberry followed by dithane M-45 (0.02%) and decolin (0.02%).

Singh *et al.* (1990) reported control of leaf spot caused by *Alternaria brassicae* under field conditions by 7 fungicides, namely emisa-6, bavistin, captafol, cumin-L, difolatan, dithane M-45 and dithane Z-78 and suggested economically viable control measures acceptable to farmers.

Chauhan and Joshi (1990) reported that the efficacy and persistence of 14 plant extracts and carbendazin as mango fruit dip treatments were compared in controlling mango fruit anthracnose (caused by *Colletotrichum gloeosporioides*). Carbendazin (0.05%) was the most effective control treatment. Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for 72 weeks when fruit were inoculated and were significantly batter than the other plant extracts tested. Caster oil (5%), Eucalyptus oil (1%), Garlic bulb, Zingiber, Mango, Turmeric and Lantana leaves also significantly controlled the disease. Persistence was max with carbendazil (0.05%) even in the pulp

followed by castor oil (10%), garlic bulb and ardusi leaves. Carbendazil leaf resides in the pulp but plant extracts only persisted in the pericarp.

Bhardwaj and Thakur (1991) reported that field experiments, during 1989 and 1990, revealed that applications of fungicides viz., mancozeb (75wp) at 0.25 %, carbedazim (50wp) at 0.1 % and captafol (80wp) at 0.25 % significantly reduced the severity of leaf spots and pod blight in mung bean (*Vigna mongo* cv. PDU1) caused predominantly by *Cercospora cruenta* and *C. canescencs* and in association with *Ascochyta phaseolorum* and *Colletotrichum dematium* f. sp. *trumcatum*.

Ahmed *et al.* (1991) reported that of the eight fungicides evaluated, dithane M-45 (mancozeb) gave the best control of anthracnose (*Colletotrichum gloeosporioides*) followed by Bordeaux mixture. They found that sooty mould (*Capnodium ramosum*) was controlled best by Bordeaux mixture and thiovit.

Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *Botryodiplodia theobromae, Fusarium oxysporum, Helminthosporium spiciferum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum*. They used some medicinal plants viz., *Calotropis procera, Vitex negundo, Thuja orientalis, Argemone mexicana, Achyranthes aspera, Datura fastusa* and *Ricinus communis* and observed good control against these pathogens. Of the 11 leaf extracts, those of *Azadirachta indica* and *Occimum sanctum* were most effective in controlling the fungi.

Sharma and Gupta (1994) reported that 6 fungicides namely carbendazim bitertanol, traidimefon, trideorph, triforine and thiophenate methyl significantly inhibited spore germination of *Podosphaera leucotricha* and reduced germ tube length. The maximum inhibition (94%) was obtained in triforine and minimum (72%) in thiophanate methyl at 500ppm. Antisporulant activity of the fungicides was observed upto 14 days in all except carbendazim and bitertanol, where there

was sporulation upto 21 days. In the field bitertanol followed by carbendazim and triforine provided the best control of mildew.

Biswas *et al.* (1995) reported that the effects of 10% alcoholic water extracts of fresh plant parts from twenty (20) different species were studied on the development of powdery mildew *Pyllanctinia corylea* (Pers. Karst.), leaf spot (*Pseudocercospora mori* Hara., Deignton) and leaf rust (*Cerotelium fici* Cast., Arth.) diseases in mulbery during 1992 - 93 and 1993 - 94. The pooled data of these two years revealed that extract of *Adhatoda vasica* was the most effective in decreasing the severity of all the diseases followed by extracts of *Azadirachta indica*, *Launea coromandelica* and *Oxalis corniculata.* Which significantly minimized two diseases, namely, powdery mildew and leaf rust, while those of *Celosia argentia* and *Eupatorium odoratum* reduced leaf spot and leaf rust diseases. Extracts from several other plant species exhibited ability to reduce either leaf rust or powdery mildew disease.

Alam *et al.* (1999) reported the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found that redomil, dithane M-45, cupravit, bavistin and rovral proved to be the most effective against *A. tenuis* when immersed for 5 to 30 minutes at 500 to 2500 ppm concentrations.

Alam *et al.* (2000) reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found bavistin, dithane M-45 and tilt were the most effective fungicides. They stated that at 500 to 2500 ppm and 1/10 to 1/1000 ml concentrations were most effective on the fungus after 5 to 30 minutes immersion

Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling purple blotch of onion seed-crop and observed that combined application of rovral 50wp @ 0.2% + redomil MZ-72 @ 0.2 % gave the best control of purple blotch and maximum seed yield of onion followed by individual

application of rovral 50wp @ 0.2 % and score 250EC @ 0.05 % when sprayed at an interval of 15 days.

Alam *et al.* (2002) reported the *in vitro* inhibition of conidial germination of *Colletotrichum gloeosporioides* Penz. and observed that dithaneM-45, rovral, thiovit and suncozeb were most effective against *C. gloeosporioides* after 5-30 minutes immersion at 0.05%-0.25% concentration.

Alam *et al.* (2002) reported the antifungal activities and (*in vitro*) inhibition of spore/ conidial germination of four fungi viz., *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae* was tested using the extracts of different parts of *vinca rosea* and *Azadirachta indica*. They observed that *Vinca rosea* root and *A. indica* (Leaf, root and seed) extracts showed good (100%) inhibition results on *B. sorokiniana* and *R. artocarpi* when it was immersed from 5-30 minutes at 5:1.25 ml (w/v) concentration.

Chapter 3

Materials and Methods

MATERIALS AND METHODS

3.1 SURVEY ON THE DISEASE:

Jute (*Corchorus capsularis* L) cultivation suffers from problems due to pests and diseases. Sometimes, serious fungal diseases limit production of jute. Black band disease of jute is one of the most important diseases, which results in premature falling of leaves and destroys jute fibres. Survey work and sample collection have been done in the Northern part of Bangladesh. The primary aim of the investigation was to ascertain the extent of damage by the causal organism of black band disease of jute.

During the present study, different diseased samples and healthy jute plant parts were brought in the laboratory for different pathological test. Fifteen jute fields were randomly selected for the investigation of black band disease in the present study from June-October 1999, 2000 and 2001. The symptoms of the disease appear at the mature stage of jute plants. The disease is caused by *Botryodiplodia theobromae* Pat. and develops a dense black band around the stem at about 2-3 feet above the ground level. Such affected plants ultimately loose all leaves and remain in the field as dry black stems (**Plate 1A and 1B**). The bark of the main stem splits longitudinally at the later stages of the disease development. The fibres turn brown and dry; the pathogen produces minute, spiracle black pycnidia on the blackend stem. Superficially these pycnidia are confused with those of the stem rot. However, on rubbing hands up and down such a stem the fingers become black due to blackish pycnidiospores formed inside the pycnidia. The infected areas subsequently increase in dimensions. During the moist weather these areas extend rapidly and gradually infected the stem. Both healthy and infected plants were counted and their percentages were determined to assess the disease incidence.

Disease incidence was recorded by adopting the grading formula of Siddaramaiah *et al.* (1978). The percentages of infected plants were calculated by the formula given below.

$$\text{Percentage of infected plants} = \frac{\text{Total No. infected plants}}{\text{Total No. of plants observed}} \times 100.$$

3.2 PATHOLOGICAL STUDIES:

Following standard phytopathological methods (Booth, 1971), the pathogen was isolated from the transitional zone of healthy and infected tissues on 2% PDA medium. Bacterial contamination was avoided by incorporating 50% lactic acid just before pouring to the plates at 45°-50°C temperatures. The inoculated plates were incubated for 13 days at 28±2°C and examined periodically. Pure cultures were transferred to test tube slants containing PDA medium. Stock cultures were revived at intervals of two months. Pure culture of the fungus (*Botryodiplodia theobromae* Pat.) responsible for development of black band disease was used as the material of this study.

3.2.1 Collection of Disease Sample:

For experimental purposes randomly selected jute plant parts of different sampling sites were used. Diseased plants were collected from selected areas for the isolation of pathogen. Collected samples were separately packed in polythene bags, labelled properly with date for future references. After collection, the samples were brought to plant pathology laboratory, Department of Botany, University of Rajshahi.

3.2.2 Method of Sterilization and Incubation:

After collection, the diseased materials of jute plant parts (leaf, stem, root etc) were brought to the laboratory and diseased parts were then cut into small pieces (2-5cm) and selected for surface sterilization. The materials were washed in running tap water, dipped in 0.1% mercuric chloride ($HgCl_2$) solution contained in a petridish for 1-2 minutes by rotating the sample frequently with a needle. These were then given several washes in sterilized distilled water to remove mercuric chloride from the materials. Excess water from the surface of the materials was removed by gently pressing them between two flaps of previously sterilized filter papers. The materials were removed with a flamed forceps and placed in a humid chamber. The materials incubated under this condition were found to produce profuse conidiophores and conidia of the fungus.

3.2.3 Isolation of Pathogen:

Isolation of pathogen from different diseased parts of the host plant noted above was made following either (a) by direct method or (b) by plating method.

3.2.4 Identification of Isolated Fungus:

The fungus isolated from infected tissues of stem on host plant has been described above. The fungus was transferred to PDA slants and sub-cultured on PDA plates for identification. The fungus was identified with the help of keys outlined by Sutton (1980).

3.2.5 Pathogenecity Test:

i) **Raising of host plants:** For pathogenecity test, the jute plants were grown, in earthen pots (9 inches) containing loam soils. Five to ten seedlings were raised in each pot for *Corchorus olitorius* L. The host of jute was inoculated with a dense conidial suspension of *Botryodiplodia theobromae* on the jute stem by artificial inoculation.

ii) **Preparation of conidial suspension**: Sterilized distilled water was used for the preparation of conidial suspension. Conidia were washed in sterilized distilled water separately, collected from 14-16 days old culture of *Botryodiplodia theobromae* grown on PDA. At first, 20 ml of sterilized distilled water was added on a culture plate and was shaken well to remove maximum number of conidia from it. Using about 4-5 plates a total of 100ml of conidial suspension was prepared for each culture. Before being used the density of conidia in water was adjusted to 40×10^4 - 50×10^4 ml from haemocytometer count.

iii) **Method of inoculation**: Seedling of jute plants were inoculated by spraying a conidial suspension with an atomizer. The inoculated plants were covered with polythene bags and incubated at 28±2°C for two days. Pot plants were then transferred to the garden of third science building. Water was added every day to keep them under moist condition after removing the polythene bags. Observation was made after 10-12 days. When the stem of the host plants had developed characteristic lesion, compared with typical symptoms recorded before under field conditions.

iv) **Re-isolation of pathogen:** The artificially infected stem having developed symptoms was collected. Isolation of the pathogen was made following the usual procedure. In most of the cases, pathogen was successfully isolated from infected plants on PDA plates. The morphological characters of the reisolated pathogen were compared with the original isolates.

3.2.6 Preparation of Different Culture Media:

The following culture media were used for laboratory tests.

a) Potato Dextrose Agar (PDA) Medium:

Peeled and sliced potatoes	200 gm
Dextrose	15 gm
Agar	20 gm
Distilled water	1000 ml

b) Richard's Medium:

Sucrose	50 gm
Potassium nitrate (KNO_3)	10 gm
Potassium hydrogen phosphate ($K_2HPO_4,7H_2O$	5 gm
Magnesium sulphate ($MgSO_4,7H_2O$)	2.50 gm
Ferric chloride ($FeCl_3$)	0.02 gm
Distilled water	1000 ml

c) Czapek's Medium:

Sucrose	30 gm
Sodium nitrate ($NaNO_3$)	2 gm
Potassium dibasic phosphate ($KH_2PO_4, 7H_2O$)	1 gm
Potassium chloride (KCl)	0.50 gm
Magnesium sulphate ($MgSO_47H_2O$)	0.50 gm
Ferrous sulphate ($FeSO_4. 7H_2O$)	0.01 gm
Distilled water	1000 ml

d) Paper Chromatography (PCM) Medium:

Chromatography paper	15 gm
Ammonium nitrate (NH_4NO_3)	1 gm
Magnesium sulphate (($MgSO_47H_2O$)	0.5 gm
Potassium Di-hydrogen phosphate ($KH_2PO_4, 7H_2$	1 gm
Yeast extracts	1 gm
Distilled water	1000 ml

e) Sabouraud's Medium:

Glucose	20 gm
Agar	20 gm
Peptone	10 gm
Distilled water	1000 ml

f) Fertilizer Medium:

Fertilizer	As necessary (gm)
Peeled and sliced potatoes	200 gm
Agar	20 gm
Distilled water	1000 ml

g) Cowdung Medium:

Cowdung	As necessary (gm)
Agar	20.00 gm
Distilled water	1000 ml

h) Oil-cake Medium:

Oil-cake	As necessary (gm)
Agar	20.00 gm
Distilled water	1000 ml

The liquid media were solidified with the addition of 2% agar, wherever required. The media were prepared according to Rangaswami (1993).

All solid media were autoclaved at 15 lb/inch^2 pressure for 20 minutes. Incase the medium contained any substance liable to decomposition or denaturation, it was subjected to fractional sterilization for three successive days.

Garrett's (1936) agar disc method was used for inoculation, except where otherwise stated. The inoculum was taken from 14-15 days old culture and size of the inoculum was the same in all cases. After inoculation the culture was incubated at 28±2°C for 15 days for mycelial growth and conidia formation.

3.3 BIOLOGICAL TEST OF FUNGUS:

3.3.1 Method of Studying the Effect of Temperature on Conidial Germination:

To study the effect of temperature on spore germination the following technique was followed. Spores were placed on cover slips coated with glucose-potato-agar. These were incubated at the selected temperature of 10°, 20°, 25°, 30°, and 35°C in petridish moist chamber. Cover slips were removed periodically, the spores killed on a drop of lactophenol and germination percentages were determined by microscopic observation.

3.3.2 Method of Studying the Effect of Humidity on Conidial Germination:

Different percentage of humidities within the range of '0' to '100' were maintained inside dessicators by filling the bottom of the dessicators with equal volume of different mixture of sulphuric acid and water as described by Buxton and Melanby (1934).

To study the effect of humidity on spore germination modified Bonner's (1948) technique was used. To maintain the desired level of relative humidities Buxton and Mellanby's (1934) method was followed. Clean dry cover slips were loaded with conidia by pressing them on the surface of a 15 days old culture grown in 0.1% glucose agar. On this medium, the fungus sporulated through the production of conidia. This method of loading cover slips provided an even distribution of conidia and did not result in clumping, which was a problem encountered with aqueous suspension. The cover slips, with adhering spores, were placed in a dessicator over night to kill the mycelial fragments and to remove excess moisture before they were suspended over sulphuric acid and distilled water solution in petridish and incubated at 28±2°C.

3.3.3 Method of Studying the Effect of pH on Conidial Germination:

To study the effect of pH on conidial germination the following method was followed. Conidia from the pycnidia of the PDA medium were taken and conidial suspension was made different range (2, 3, 4, 5, 6, 7, 8, 9 and 10) of pH. 5 ml of those suspensions were taken in watch glass and kept at room temperature for 6 hours. After that period, a drop of incubated conidia taken on slide and one drop of lactophenol was added to kill the conidia. The slides were examined under microscope for note the rate of germination. Percentages of germination were recorded and mean values were calculated.

3.3.4 Method of Studying the Effect of Different Substances on Conidial Germination:

For the test of conidial germination, distilled water, pond water, tap water, sterilized water, river water, glucose, sugar different concentrations and nitrogen salts (1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) and cowdung (1:5,1:10, 1:15, 1:20, 1:25 and 1:30 w/v) were used as different testing media. Conidia were taken from pure cultures in PDA medium and suspensions were prepared separately in different test substances. These suspensions were (5 ml) taken in small watch glass separately and were kept at room temperature for different hours (2-24 hours). After that period, a drop of incubated conidial suspension of different substances was taken on separate slide. A drop of lactophenol was also put in the conidial suspension in the slide and such slides were examined under high power for recording the percentage of germination. Germination percentages were recorded in three replications for each and mean values were calculated.

3.3.5 Method of Studying the Effect of Mycelial Growth on Different Solid Media:

For experiment with solid media, 20 ml of the media were poured after sterilization under aseptic condition, in sterilized 90 mm petridish and inoculated as described earlier. The measurement of radial growth of the colony were taken of intervals of 24 hours and expressed in mm. Increase of circular colonies, the diameter of the colony was measured in two direction at right angles to each other, whereas in case of irregular colonies the measurement was taken along the longest and the shortest directions and the average was taken as the growth of the colony (Brown, 1923).

On the other hand, PDA, PCM, Sabouraud's, Czapek's, Richard, and different concentrations of cowdung (5, 10, 15, and 20%) media were used for the observation of growth, dry weight and sporulation of *B. theobromae*. For mycelial growth tested of *B. theobromae* in different concentrations of fertilizer (Urea, Pottus, Gypsum, Boron, Sulpher, and Zinc) and oil-cake (Mustard and Lin seed) media were used.

3.3.6 Method of Studying the Effect of Different Concentrations of Glucose, $NaNO_3$ and KNO_3 on PA (potato-agar) Medium for the Formation of Pycnidia and Mycelial Growth:

Method of studying the effect of different concentrations of glucose, $NaNO_3$ and KNO_3 on PA (potato-agar) medium for the formation of pycnidia and mycelial growth the following method was followed. Different concentrations of glucose, $NaNO_3$, and KNO_3 (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) were added in PA medium. 20 ml of the medium was poured after sterilization at 90 mm petridishes. 5mm diameter mycelial block from seven days old culture of *B. theobromae* was inoculated in the center of the medium. The plates were incubated at 28±2°C for 15 days and observation was made on the mycelial growth and formation of pigments and pycnidia.

3.3.7 Method of Studying the Effect of Leaf Extracts Media of Some Plants on the Mycelial Growth of *B. theobromae.*

The leaf extract medium was prepared as follows: leaves, 200g (decoction); agar, 17g, distilled water, 1L. The leaves were washed thoroughly, cut into bits and streamed in 500 ml distilled water for 30 min, strained through a muslin cloth and the decoction was added to the melted agar (which was steamed in 500 ml of water) and the volume was made up to 1L. Media were autoclaved at 15 p.s.i. for 20 min. Equal quantities of the medium was poured in each petriplate of 9 cm diameter. After solidification, triplicate plates of each leaf extract agar medium were inoculated by using 5 mm inoculums with agar disc, which was cut from the margins of actively growing colonies of this pathogen on PDA. Plate of PDA served as control.

3.3.8 Method of Studying the Effect of Different Solid Media on Mycelial Dry Weight and Sporulation:

For determining the dry weight of the fungus, inoculations were made in 25 ml liquid medium apportioned in 90 mm petridishes and incubated for 5 days when mycelial growth had reached maximum. After this period, the entire fungal mat was harvested by filtering over previously dried and weight Whatman's filter paper no 42. It was then washed with distilled water and dried to constant weight at 65°C. Before weighting, the filter paper were transferred to a dessicator and allowed to cool. They were subsequently weighted in a chemical balance. Tubes containing fused calcium chloride were kept inside the balance absorbed atmosphere moisture while the weighting was in progress. The weight of the mycelial mat was calculated by deducting the dry weight of the filter paper from the final weight.

To study effect of media on sporulation following method was followed. Test tube slants of different media were inoculated with a standard amount of the same spore suspension and incubated at 28±2°C. After 15 days of incubation, the

slants were flooded with 3 ml of sterile water and the conidia loosened by the gently massaging the surface with a sterile loop. The conidia were counted with the help of haemocytometer under low power microscope. The growth of mycelium and production of spores were studies on solidified media.

3.3.9 Method of Studying the Effect of Temperature and Light on the Mycelial Growth and Sporulation:

Stock culture of *Botryodiplodia theobromae* was maintained on PDA plates. Plates of PDA and cowdung media were inoculated with 5mm culture discs and was cut with a sterile cork borer from the advancing margin of colonies and kept in PDA and cowdung plates. Carbon paper was used to wrap the petridishes to create darkness. Fluorescent lamp was used for light treatment. The inoculated plates were incubated under fluorescent lamp (at one foot height), at temperature 10, 15, 20, 25, 30, 35, 40 and 45°C. The inoculated plates were subjected to light exposure as follows: (a) continuous light, (b) 16 hours light and 8 hours darkness, (c) 8 hours light and 16 hours darkness, (d) alternating 12 hours light and darkness, (e) alternating 24 hours light and darkness, (f) continuous darkness and (h) room condition. Colony diameter was used as the basis of growth. All cultures were examined after 3 days for growth and for sporulation after fifteen days of incubation under various conditions as described above. A haemocytometer was used for counting the degree of sporulation, following Tuite, 1969.

3.3.10 Viability of conidia of *Botryodiplodia theobromae* in storage at room temperature:

To test the conidial viability of *B. theobromae,* culture was stored at room temperature. Conidial germination was tested in every month by removing the stored conidia from the incubation and allowing then to germination in 1.5% glucose solution. Germination was recorded under microscope and percentage of conidial viability was calculated.

3.4 CONTROL MEASURE:

3.4.1 Method of Studying Fungicidal Effect *in vitro*:

The evaluation of fungicides in the laboratory was conducted by using Forsberg's (1949) technique with slight modification. Thick cotton threads were cut in to pieces of 2 cm long and were sterilized. They were then spread on the surface of sterilized PDA medium contained in petridishes. The petridishes were incubated with the pathogen and incubated at 28±2°C. When the threads were thoroughly covered with mycelium and spores, they were removed with the help of sterilized forceps. The threads were put in triplicate in fungicidal solution or suspension of different concentrations, made in sterile water in different time period. The threads were then transferred to 2% agar plates after removing excess fungicides on sterilized filter paper. If the fungus on the threads treated did not grow in the agar plate it showed that the fungicide was effective.

3.4.2 Method of Studying Plant Extraction as Fungicides from of Root, Seed and Leaf Tissues of Different Plants in Alcohol:

Extraction of fungicides from root, seed, bark and leaf tissues in alcohol was done following the method described by Mahadevan and Sridhar (1982).

Five gram tissues were cut into pieces and immediately plunged in boiling ethyl alcohol in a beaker and allowed to boil 5-10 minutes, used 5-10 ml of alcohol for every gram of tissues. The extraction was done on top of a steam bath. The extraction was cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a paste and then passed through two layers of cheesecloth and re-extracted the ground tissues for 3 minutes in hot 80% alcohol, using 2-3 ml of alcohol soluble for every gm of tissues. The second extraction ensured complete removal of alcohol soluble substances and cooled and passed through cheesecloth. Cooled both extracts and filtered through Whatman's no. 1 filter paper.

The volume (10ml) of the extract was evaporated on steam bath to dryness and 1.25 ml of distilled water was added for five grams of tissues and the extracts were used as fungicides. Conidia from the culture on PDA plates were taken and conidial suspensions were made separately with different plant extracts. These suspensions (1.25 ml) were taken in sterilized watch glass and were kept at 30±2°C for 5-30 minutes. After that period, a drop of treated conidial suspension with different plant extracts was taken on separate slides for 24 hours of incubation. After incubation period, one drop of lactophenol was added on slide to kill the conidia. Percentages of conidial germination inhibition were recorded and mean values were calculated following, Ashrafuzzaman, 1976.

3.4.3 Method of Studying the Effect of Smoke on the Conidial Germination:

The evaluation of fungitoxicity of smoke in the laboratory was conducted by using Parmeter's (1975) technique with a modification. Rice straw, wheat straw, tobacco leaf and dhup were burnt in a metal pot with a cover filtered with rubber tube, the resulting smoke was cooled to ambient temperature by passage through the rubber tube. The cooled smoke was introduced into petriplates containing PDA with fungal colony. The petriplates containing cultures were placed for varying lengths of time (5, 10 and 15 minutes) in the smoke chamber and exposed to dense rice straw, wheat straw, tobacco leaf and dhup smoke.

Aqueous conidial suspension of the pathogen was placed on slides previously exposed to smoke of rice straw, wheat straw, tobacco leaf and dhup smoke was incubated in a moist chamber for 24 hours.

3.4.4 Method of Studying the Effect of Ginger and Neem Extracts with Cowdung on the mycelial Growth:

To study the effect of ginger and neem extracts with cowdung against the growth of *B. theobromae,* experiment was set up in laboratory following the methods of Brown (1923). 20ml of media (20% ginger +15% cowdung and 20% neem+15% cowdung) was poured after sterization under specific condition in sterilized 90mm petridishes and inoculated as described earlier.

3.5 ANALYSIS OF DATA:

Statistical Analysis:

For evaluating and interpreting the results, collected data were analysis following the biometrical technique developed by Mather and John (1971) based on the mathematical models of Fisher *et al.* (1932). Statistical analysis of data given as percentage was carried out from angular transformed values.

Variance Analysis:

The analysis of variance was done for sorting out the variance due to different sources and for tasting the significance among the sources. Variance analysis for each character was carried out separately on mean value of ten cultures in each of the repeated experiment.

Mean square in the analysis of variance were calculated as follows:

$$\text{Sum of square (SS)} = \sum x^2 - \frac{\left(\sum x\right)^2}{n}$$

$$\text{Mean square (MS)} = \frac{\sum x^2 - \left(\sum x\right)^2 / n}{n-1}$$

Where,

Σx =Sum of x.

n =Sample size.

Variance Ratio Test (F- test):

F- test was carried out for test of significant among the variance within a source.

$$\text{F value} = \frac{MS}{EMS}$$

Where, MS = Mean square value of specific source of variance.

EMS=Error mean square.

Test of Least Significant Difference (LSD):

LSD were determined, whenever, the calculated 'F' value were significant at 5% level.

To test the least significant differences the following formula was used.

$$\text{LSD at 5\% level} = \sqrt{\frac{2 \times EMS}{r}} \times t \text{ at 5\% level.}$$

Here,

EMS = Error mean square

r = Number of replications.

5% level t = value of at 5% significant level from tabulated t table.

Correlation:

To determinate the relationship between two variables the following formula was used.

$$r = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2}$$

x = Variable No. 1

y = Variable No. 2

$\bar{x}$ = Mean of x variable.

$\bar{y}$ = Mean of y variable.

Chapter 4

Results

RESULTS

4.1 Field Investigation of Black band Disease of Jute.

Black band disease of jute has been recorded from the selected places (Rajshahi, Naogaon, Natore, Bogra, Gaibanda, Joypurhate, Dinajpur, Nilphamari, Thagorgoue, Rangpur, Kurigram, Lalmonirhat, Pabna and Sirajgong districts) in the Northern areas of Bangladesh during the crop season in 1999 to 2001 (June to October). It is evident form Table-1A that the highest incidence of black band disease of jute was found to be in Naogaon district during the crop seasons in 1999 & 2000 and Gybhanda in 2001. It is also evident from the observation that disease incidence always increased with the advanced of time and highest disease incidence was recorded in the month of September in all surveyed places. Meteorological data showed that high humidity, rainfall and high temperature is more favorable for the development of black band disease of jute (Table-1B). In this experiment, it is clear from Meteorological and *in vitro* data of temperature results are very closely related to each other. On the other hand, in case of relative humidity, it was showed a little difference both *in vivo* and *in vitro* results (Table-2 and Table-3). However, the pattern of disease incidence of different places showed more or less same with little variation.

Analysis of variance on percentage of disease incidence of different month in different crop season show that the items district, month and district × month is significantly different in every season. Analysis of variance on percentage of disease incidence (average) of different crop season in different districts also show that the items crop season and district are significantly different.

4.2 Symptoms of "Black band" Disease of Jute.

The disease develops a dense black band around the stem at about 2-3 feet above the ground level. Such affected plants ultimately loose all leaves and remain in the field as dry black stems. The bark of the main stem splits longitudinally at the later stages of disease development. The fibre turn brown and dry, the pathogen produces minute, spherical black pycnidia on the blacken stem. Superficially these pycnidia are confused with those of the stem rot. However on rubbing hands up and down such as stem, the fingers become black due to blackish pycnidiophores formed inside the pycnidia (**Plate 2A, 2B and 2C**).

4.3. Morphology of the Causal Organism (*Botryodiplodia theobromae* Pat.) and its Taxonomic Position.

Colony was cottony-gray, white to dark, with always black on PDA reaching a diameter of 9.0 cm culture after15 days at 28±2°C. Aerial mycelium was grey-white becoming dark colored with maturity (**Plate 3A and 3B**).

Mycelium immersed or superficial, branched, septate, dark chocolate brown. Conidiomata eustromatic, immersed or superficial, separate or aggregated and confluent, globose carbonous, dark brown, uni-or multicolor, wall of dark brown, thick walled textura angularis, paler and thinner towards the conidiogenous region, often with dark brown superficial hyphae over the surface (**Plate 4A**).

The pycnidia lie aggregated in botryose (grape-like) clusters; hence the name *Botryodiplodia.* The pycnidia are thick-walled, dark-brown and ostiolate. Conidiophores are cylindrical hyaline and show distinct apical annulations. Conidia ellipsoid or ovoid, 2-celled, dark-brown, with characteristic striations at maturity. Young conidia are 1-celled and hyaline.Conidia were single and two celled. Single celled conidia were hyaline, thin and bitunicate and measured 18.2-27×9.9-11.1 µ. Double celled conidia were dark, thick, longitudinally striated and measured 24.0-28.2× 10.4-14.1 µ (**Plate 4B**).

Taxonomic position according to Anisworth, 1966.

Division: Eumycota

Sub division: Deuteromycotina

Form class: Coelomycetes

Form order: Sphaeropsidales

Form family: Sphaeropsidaceae

Form genus: *Botryodiplodia*

Form species: *B. theobromae*.

4.4 GERMINATION OF CONIDIA

Most of the fungi multiply asexually with their conidia. This conidia germinate to produce their plant body i.e. mycelium. The role of conidial germination depends on different environmental and physiological factors. To asses the role of these factors for conidial germination of this fungus, present observation have been carried out.

4.4.1 Mode of Germination

The germinating conidia of *B. theobromae* are marked with a swelling and the inner cell contents become distinct. After two to three hours of swelling, the inner layer of the wall is drawn and acts into a small tubular protuberance, which ultimately formed a germ tube (**Plate 4C**).

PLATE - 1

Photograph of Healthy and Infected jute plants.

A. Healthy jute plants.

B. Infected jute plants.

PLATE - 1

PLATE - 2

Symptoms showing Black band disease of jute at different stages.

A. Mature stage.

B. Early stage.

C. Pycnidia formed on plants.

PLATE - 2

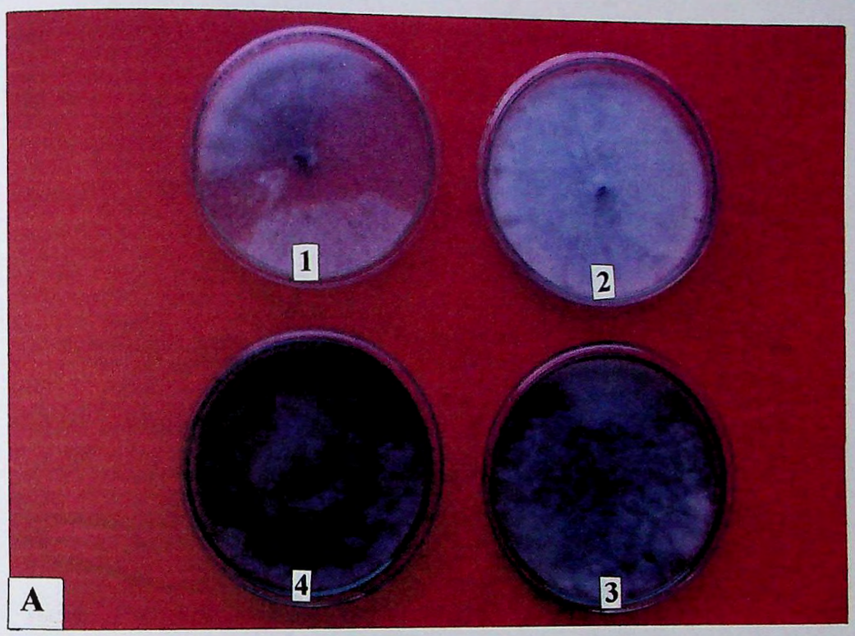
PLATE - 3

A. Showing different mycelial colour.

(1) and (2) White cottony mycelia.
(3) Gray cottony mycelia.
(4) Black mycelia.

B. Showing formation of pycnidia both oily and cluster stages.

PLATE - 3



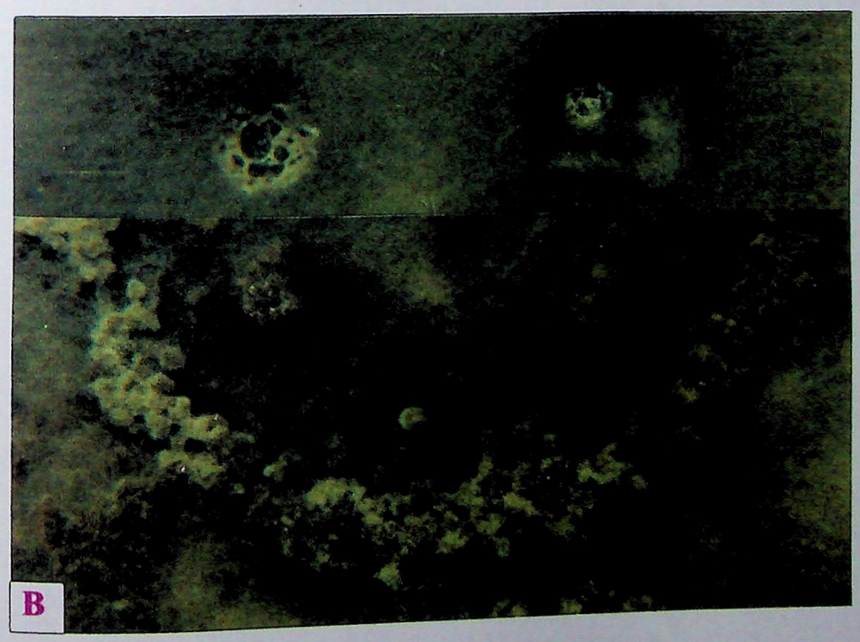


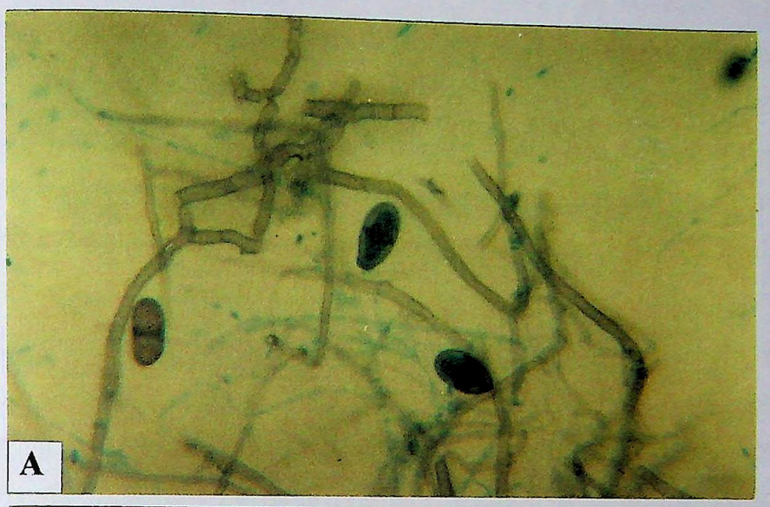
PLATE-4

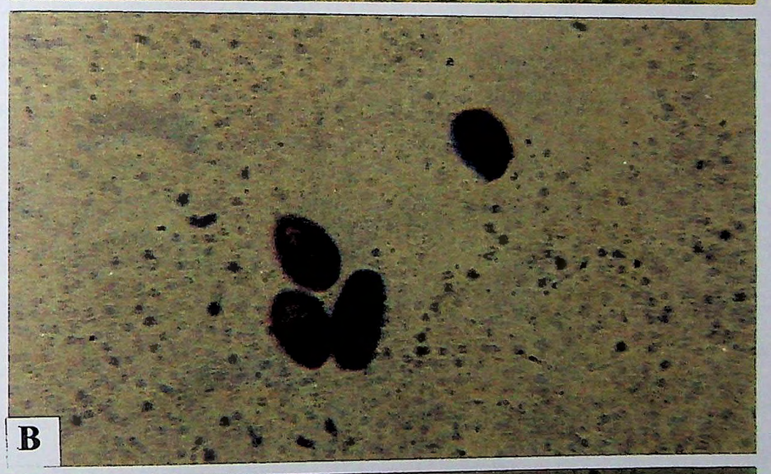
A. Mycelia with conidia.

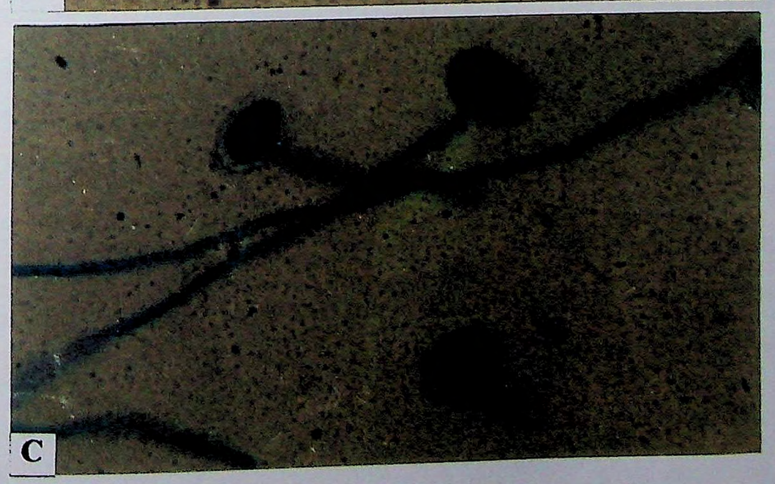
B. Conidia of *B. theobromae*.

C. Germinating conidia.

PLATE - 4







4.4.2 Factors Affecting Germination

Environmental and nutritional factors influence greatly the germination of conidia. Temperature, humidities, pH and nutrition are important factors for conidial germination. The conidia of *B. theobromae* were allowed to germinate separately under different temperatures, humidities, nutrients and pH as described under materials and methods and observation were recorded.

4.4.2.1 Effect of Temperature

In the present investigation, conidial germination of *B. theobromae* occurred over the temperatures range of 15°-40°C and the optimum temperatures range from 25°-30°C on 1.5% of glucose and sucrose solution respectively (Table-2). In case of 1:15 (w/v) cowdung solution, it was showed at 35°C. Germination of conidia was initiated with in 4 hours incubation in every cases and maximum percentage of conidial germination (90, 97, 98%; 88, 96, 98% and 35, 69, 78%) was attained in 6-10 hours in glucose, sucrose and cowdung solution respectively. It was observed from Table-2 that 100% conidial germination occurred both in glucose and sucrose solution at 25°C and 30°C after 24 hours incubation. In case of cowdung solution it was recorded at 35°C after 8-24 hours incubation. In every cases, no conidial germination was observed at 10°C and 45°C even after 24 hours of incubation. Marked variation of conidial germination was recorded in cowdung solution than that of glucose and sucrose solution, even though the temperature remain same in every cases in this experiment.

Range of correlation (r1) values 0.0274 to 0.0940, -0.272 to 0.0601 and 0.0261 to 0.0957 for glucose, sucrose and cowdung respectively indicate that there was no highly significant reationship between temperature and conidial germination. But correlation (r2) values 0.7776, 0.7935 and 0.8002 for glucose, sucrose and cowdung respectively indicate that there was highly significant reationship between incubation period and conidial germination. Analysis of

of pH level (2, 3, 4, 5, 6, 7, 8, 9 and 10). The highest germ tube length was found to be measured 6.40-198.00 at pH - 6.

Correlation (r5) value 0.2752 indicates that there was a significant relation between pH level (2-10) and percentage of conidial germination. Analysis of variance on conidial germination of *B. theobromae* in different pH shown that the items pH was significan at 5% level of probability. LSD value also indicates same result.

4.4.3 EFFECT OF NUTRITIONS

4.4.3.1 Effect of Different Concentrations of Glucose and Sucrose Solution on Conidial Germination

From the present study, effect of different concentrations of glucose and sucrose solution on conidial germination of *B. theobromae* after 2-12 hours are shown in Table-5. In both the cases, germination was initiated within 4 hours of incubation. After 12 hours of incubation, the highest (100% and 99%) conidial germination was recorded in 1.5% glucose and 1% sucrose solution. The concentration 1.5% and 1% (optimum concentration) of glucose and sucrose respectively resulted better germination as compared to other concentrations of glucose and sucrose solution used in this study. In every cases, germination was recorded the lowest (87% and 81%) in 3.5% concentration of glucose and sucrose solution. Rest of the concentrations of glucose and sucrose were showed intermediary effect on the conidial germination. It is evident from Table-5 that the conidial germination was decreased with the decrease or increase of concentrations of glucose. However, in cases of sucrose solution, the rate of conidial germination was alaways decreased. After 10 hours of incubation, the length of germ tube was measured to be 39.83 μ - 108.75 μ and 32.12 μ - 96.37 μ in glucose and sucrose solution respectively. Length of germ tube was measured the higher on glucose solution than that of sucrose solution.

Range of correlation (r6) values –0.642 to 0.1153 and –0.9943 to –0.9559 for glucose and sucrose solution indicate that there was negative significant relationship between concentrations and conidial germination. Correlation (r7) values 0.9329 and 0.9315 for glucose and sucrose solution respectively indicate that there was highly significant relationship between incubation period and conidial germination. Analysis of variance on conidial germination of *B. theobromae* in different concentrations of glucose and sucrose solution shows that the items incubation period, concentration and incubation period × concentration were significant in every cases. LSD values also indicate significant different in conidial germination for every cases.

4.4.3.2 Effect of Different Concentrations of Cowdung Solution on Conidial Germination

In the present investigation, effects of different concentrations of cowdung solution on conidial germination of *B. theobromae* after 2-12 hours are shown in Table-6. Among the concentrations used, 1:1.5 (w/v) concentration gave the highest germination (100%) of conidia. Germination of conidia was initiated on cowdung solution after 4 hours of incubation and germinated in all the concentrations within 12 hours. It is evident from Table-6 that rest of the concentrations (1:5, 1:10, 1:15, 1:20, 1: 25 and 1:30) had intermediary effect on conidial germination. In the present observation, it may be concluded that *B. theobromae* can freely grow on cowdung media, 1:15 (w/v) cowdung concentration showed 100% conidial germination after 12 hours of incubation. The length of germ tube (51.4μ-α) was measured on cowdung solution after 12 hours of incubation. This observation indicates that conidial germination was faster in cowdung solution than that of glucose and sucrose solution.

Ranges of correlation (r8) values –0.6919 to –0.3964 indicate that there was negative correlation between conidial germination and concentration. Correlation

(r9) values 0.8993 to 0.9943 indicate that there was highly significant relationship between conidial germination and incubation period. Analysis of variance on conidial germination of *B. theobromae* in different concentration of cowdung solution shows that the items incubation period, concentration and incubation period × concentration were significant. LSD value also indicates significant different in conidial germination.

4.4.3.3 Effect of Different Types of Water on Conidial Germination of *B. theobromae* after 8 hours of Incubation at 28±2°C.

Effects of different types of water on conidial germination of *B. theobromae* are shown in Table-7. The results indicate that the conidial germination was better (93%) in river water than other water used. On the other hand, in rain, distilled and pond water conidial germination recorded were 90, 88 and 65%, which was better than of tap and sterilized water (44 and 54%). It may be considered from the present study, that river water contains some special nutrient supplements, which helps the germination of conidia of *B. theobromae*. Formation of germ tube length also higher in river water than that of other water used.

Analysis of variance on percentage of conidial germination of *B. theobromae* in different types of water shows that the item different water was significant. LSD value also indicates significant different of conidial germination in different water.

4.4.3.4 Effect of Different Concentrations of KNO_3 and $NaNO_3$ Salt Solution on Conidial Germination.

From the present study, effect of different concentrations of KNO_3 and $NaNO_3$ salt solution on conidial germination of *B. theobromae* after 2-24 hours is shown in Table-8. In both the cases, conidial germination was initiated with in 4 hours of incubation. After 24 hours of incubation, the highest conidial germination was recorded in 1% KNO_3 and $NaNO_3$ (48 and 88%) salt solution. 1%

concentration of KNO_3 and $NaNO_3$ resulted better germination as compared to other concentrations of KNO_3 and $NaNO_3$ salt solution used in this experiment. In every cases, germination of conidia was the lowest (13 and 40%) in 3.5% concentration of both the salt solution. Rest of the concentration of KNO_3 had poor effect and $NaNO_3$ had moderate effect on germination of conidia. In case of 2.5, 3.0 and 3.5% of KNO_3 salt solution, conidial germination started on 6^{th} and 8^{th} hours of incubation. It is evident from Table-8 that with the increase of KNO_3 and $NaNO_3$ concentrations the rate of germination was decreased. After 24 hours of incubation, the length of germ tube measured on KNO_3 salt solution was to be 51.4 – 695 μ and on $NaNO_3$ solution it was 64.25 – 835.25 μ. Length of germ tube was measured higher on $NaNO_3$ solution than that of KNO_3 solution.

Range of correlation (r10) values –0.9964 to –0.9261 and –0.9973 to -0.9904 for KNO_3 and $NaNO_3$ solution indicate that there was highly negative significant relationship between concentration and conidial germination. Correlation (r11) values 0.8331 and 0.7907 for KNO_3 and $NaNO_3$ respectively indicate that there was highly significant relationship between incubation period and conidial germination. Analysis of variance on conidial germination of *B. theobromae* in different concentrations of KNO_3 and $NaNO_3$ show that the item incubation period, concentration and incubation period × concentration were significant in every cases. LSD values also indicate significant different in conidial germination for every cases.

Table-1A: Incidence of black band disease of jute as observed and recorded in the northern areas of Bangladesh during 1999, 2000 and 2001 crop season.

District	Percentage of disease incidence											
	1999				2000				2001			
	July	August.	Sept	Average	July	August.	Sept.	Average	July	August.	Sept.	Average
Rajshahi	22	24	26	24	23	25	27	25	19	23	27	23
Naogaon	22	26	30	26	24	27	30	27	20	24	28	24
Natore	21	23	25	23	20	22	24	22	19	21	23	21
Bogra	22	23	24	23	20	24	28	24	22	25	28	25
Jypurhate	20	22	24	22	22	24	26	24	23	26	29	26
Gybhanda	23	24	25	24	21	25	29	25	24	27	30	27
Rangpur	23	25	27	25	23	26	29	26	20	23	26	23
Kurigram	21	23	25	23	22	25	28	25	21	24	27	24
Lalmonerhat	21	22	23	22	21	23	25	23	18	21	25	21
Dinajpur	20	21	22	21	20	23	26	23	18	20	22	20
Nylphamary	18	20	22	20	19	21	23	21	17	19	21	19
Thagorgoue	20	21	22	21	20	24	28	24	19	22	25	22
Pabna	22	25	28	25	23	26	29	26	30	23	26	23
Sirajgong	20	22	24	22	21	23	25	23	21	24	29	24

Analysis of variance on percentage of disease incidence of different month in 1999 crop season.

ITEMS	SS	DF	MS	F
Replication	246.8571	2	123.4286	272.4923*
District	350.3571	13	26.95055	59.49852*
Month	289.7143	2	144.8571	319.8*
District × Month	58.28571	26	2.241758	4.949112*
Error	37.14286	82	0.452962	
Total	982.3571	125	7.858857	

$LSD_{(0.05)}$ 1.095197

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on percentage of disease incidence of different month in 2000 crop season.

ITEMS	SS	DF	MS	F
Replication	236.6825	2	118.3413	242.6959*
District	323.246	13	24.86508	50.99365*
Month	651.873	2	325.9365	668.4351*
District × Month	50.34921	26	1.936508	3.971417*
Error	39.98413	82	0.487611	
Total	1302.135	125	10.41708	

$LSD_{(0.05)}$ 1.136315
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on percentage of disease incidence of different month in 2001 crop season.

ITEMS	SS	DF	MS	F
Replication	297.1905	2	148.5952	188.0096
District	686.9286	13	52.84066	66.85644
Month	608.7143	2	304.3571	385.0867
District × Month	225.2857	26	8.664835	10.96315
Error	64.80952	82	0.79036	
Total	1882.929	125	15.06343	

$LSD_{(0.05)}$ 1.446686
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on percentage of disease incidence (average) of different crop season in different districts.

ITEMS	SS	DF	MS	F
Crop season	13	2	6.5	4.567568*
District	103.6429	13	7.972527	5.602317*
Error	37	26	1.423077	
Total	153.6429	41	3.747387	

$LSD_{(0.05)}$ 2.002588
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-1B: Meteorological Data on Temperature, RH and Rainfall of three years.

Month	1999			2000			2001		
	Average Tem. (°C)	Humidity	Rainfall (mm)	Average Tem. (°C)	Humidity	Rainfall (mm)	Average Tem. (°C)	Humidity	Rainfall (mm)
January	17.96	75.94	-	17.30	75.90	004	16.78	72.38	-
February	22.15	65.08	-	19.10	67.11	047	20.15	73.39	00.40
March	26.27	62.52	-	24.73	63.72	027	25.37	52.49	008.6
April	31.66	65.09	009	28.83	72.92	136	29.91	60.83	013.4
May	29.38	66.37	144	29.08	74.22	198	28.37	83.33	207.8
June	30.29	79.28	348	29.75	81.66	244	29.05	81.33	323.8
July	29.17	86.98	349	29.73	83.23	115	29.51	85.17	336.3
August	29.06	85.55	345	29.83	85.21	190	29.99	82.33	210.4
September	28.44	85.14	502	28.55	81.22	644	29.13	81.33	096
October	27.95	76.74	155	28.18	79.83	085	27.97	84.66	185
November	24.40	81.73	-	24.28	84.49	-	24.45	82.83	00.60
December	20.34	83.64	-	19.37	85.50	-	18.97	84.49	-

Meteorological Data (Collected from Meteorological office, Shampur, Rajshahi).

Table-2: Effect of temperature on conidial germination of *B. theobromae* at 1.5% glucose, sucrose and cowdung (1:15 w/v) extract after 24 hours of incubation.

Name of Solution	Incubation Period	Percentage of conidial germination[1] in different temperatures in °C.								Length of germ tube of conidia (μ)	Correlation (r1)
		10	15	20	25	30	35	40	45		
Glucose	2	0	0	0	0	0	0	0	0	0	-
	4	0	5	15	52	71	35	8	0	0.24-6.14	0.0940
	6	0	12	35	65	90	53	12	0	2.25-20.56	0.0560
	8	0	20	48	88	97	69	15	0	7.38-56.11	0.0274
	10	0	31	56	95	98	80	26	0	26.41-72.96	0.0314
	12	0	36	62	98	99	90	33	0	38.12-98.37	0.0454
	24	0	41	75	100	100	93	41	0	α	0.0371
Sucrose	2	0	0	0	0	0	0	0	0	0	-
	4	0	2	11	48	52	27	2	0	0.25-5.85	0.0601
	6	0	6	25	62	88	45	4	0	4.47-22.28	0.0469
	8	0	16	44	85	96	66	6	0	5.51-53.38	0.0029
	10	0	22	58	91	98	74	7	0	16.24-76.63	-0.0225
	12	0	28	65	97	99	83	12	0	20.49-118.72	-0.0145
	24	0	32	77	100	100	86	16	0	α	-0.0272
Cowdung (1:15 w/v)	2	0	0	0	0	0	0	0	0	0	-
	4	0	5	11	19	25	70	0	0	25.70-231.30	0.0719
	6	0	14	18	27	35	85	0	0	25.70-295.55	0.0268
	8	0	22	30	48	69	100	4	0	36.55-346.95	0.0957
	10	0	35	43	59	78	100	9	0	43.60-398.35	0.0609
	12	0	45	56	65	85	100	15	0	43.60-424.05	0.0382
	24	0	52	66	73	90	100	22	0	α	0.0261

Correlation (r2) - 0.7776, 0.7935 and 0.8002 for glucose, sucrose and cowdung respectively.

1 – Mean of three replications.
Correlation (r1) - Correlation between conidial germination and different temperature.
Correlation (r2) - Correlation between conidial germination and incubation period.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different temperature in glucose solution.

ITEMS	SS	DF	MS	F
Replication	38.63328	2	19.31664	36.37819*
Incubation period	40585.62	6	6764.27	12738.86*
Temperature	94492.09	7	13498.87	25421.84*
Incubation period × Temperature	20208.26	42	481.149	906.127*
Error	58.40946	110	0.530995	
Total	155383	167	.930.4372	

$LSD_{(0.05)}$ 1.178053
ns= Non significan at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different temperature in sucrose solution.

ITEMS	SS	DF	MS	F
Replication	49.3926	2	24.6963	38.52417*
Incubation period	36221.81	6	6036.968	9417.165*
Temperature	93477.22	7	13353.89	20830.95*
Incubation period × Temperature	21188.32	42	504.4838	786.9526*
Error	70.5166	110	0.64106	
Total	151007.3	167	904.235	

$LSD_{(0.05)}$ 1.294401
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different temperature in cowdung extract.

ITEMS	SS	DF	MS	F
Replication	21.10319	2	10.5516	46.02251*
Incubation period	33806.07	6	5634.344	24575.11*
Temperature	83622.53	7	11946.08	52104.75*
Incubation period × Temperature	18936.9	42	450.8785	1966.58*
Error	25.21974	110	0.22927	
Total	136411.8	167	816.8372	

$LSD_{(0.05)}$ 0.774094
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-3: Effect of different level of relative humidities on conidial germination on 1.5% glucose solution of *B. theobromae* after 2 to 24 hours of incubation at 28±2°C.

Period of incubation (hours)	Germination percentage[1] of conidia in different relative humidities					Length of germ tube at 95% relative humidity	Correlation (r3)
	80%	85%	90%	95%	100%		
2	00	00	00	00	00	0	-
4	00	00	10	14	40	22.50-180.30	0.9605
6	00	11	20	41	62	25.00-210.00	0.9913
8	00	17	28	45	73	32.50-286.50	0.9809
10	00	23	36	62	88	36.60-345.20	0.9846
12	00	30	45	74	95	40.50-395.80	0.9818
24	00	35	65	95	99	42.50-430.50	0.9750
Correlation (r4)	-	0.8120	0.8922	0.8989	0.7988		

1 – Mean of three replications.
Correlation (r3) - Correlation between conidial germination and relative humidities.
Correlation (r4) - Correlation between conidial germination and incubation period.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different relative humidities in 1.5% glucose solution.

ITEMS	SS	DF	MS	F
Replication	45.311113	2	22.65556	28.54514*
Incubation period	25969.7816	6	4328.297	5453.489*
Relative humidity	36513.2733	4	9128.318	11501.33*
Incubation period × Relative humidity	9643.96463	24	401.8319	506.2928*
Error	53.9698894	68	0.793675	
Total	72226.3006	104	694.4837	

$LSD_{(0.05)}$ 1.452626
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-4: Effect of pH level on conidial germination of *B. theobromae* after six hours of incubation.

pH value	Germination[1] percentage of conidia	Length of germ tube of conidia in (µ)
2	8	4.94-102.25
3	15	5.95-110.55
4	52	6.10-145.30
5	70	6.30-165.70
6	83	6.30-196.45
7	82	6.40-198.00
8	48	6.35-162.25
9	35	6.00-156.40
10	24	4.90-125.35
Correlation (r5)	0.2752	

1 – Mean of three replications.
Correlation (r5) – Correlation between pH level and percentage of conidial germination.

Analysis of variance on percentage of conidial germination of *B. theobromae* in different level of pH.

ITEMS	SS	DF	MS	F
Replication	18.50362	2	9.251811	59.99607*
pH	7435.482	8	929.4352	6027.194*
Error	2.467311	16	0.154207	
Total	7456.453	26	286.7866	

$LSD_{(0.05)}$ 0.679739
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-5: Effect of different concentrations of glucose and sucrose solution on conidial germination of *B. theobromae* after 12 hours of incubation at 24±2°C.

Name of the solution	Period of incubation (hours)	Percentage of conidial germination[1] in different concentrations.						Length of germ tube at 2.5% concentration (μ)	Correlation (r6)
		1%	1.5%	2%	2.5%	3%	3.5%		
Glucose	2	0	0	0	0	0	0	0	-
	4	32	68	41	27	24	20	2.57-19.27	-0.6426
	6	50	89	53	43	39	34	14.13-48.83	-0.6293
	8	64	98	62	58	53	45	16.70-73.24	-0.6159
	10	70	99	75	67	61	56	39.83-108.75	-0.6037
	12	90	100	92	90	89	87	57.82-α	0.1153
Sucrose	2	0	0	0	0	0	0	0	-
	4	60	50	39	32	28	25	1.28-20.56	-0.9769
	6	87	67	55	47	41	35	6.42-50.11	-0.9559
	8	96	82	73	65	59	48	23.13-62.96	-0.9724
	10	97	94	87	79	71	67	32.12-96.37	-0.9934
	12	99	96	92	88	85	81	52.68-α	-0.9882

Correlation (r7) - 0.9329 and 0.9315 for glucose and sucrose respectively.

1 – Mean of three replications.

Correlation (r6) - Correlation between conidial germination and concentration of solution.

Correlation (r7) - Correlation between incubation period and conidial germination (average of different concentration).

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different concentration of glucose solution in different incubation period.

ITEMS	SS	DF	MS	F
Replication	80.99251	2	40.49625	5.623152*
Incubation period	58831.44	5	11766.29	1633.821*
Concentration	8500.043	5	1700.009	236.0566*
Incubation period × Concentration	2361.211	25	94.44843	13.11474*
Error	504.119	70	7.2017	
Total	70277.81	107	656.802	

$LSD_{(0.05)}$ 4.375724

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different concentration of sucrose solution in different incubation period.

ITEMS	SS	DF	MS	F
Replication	68.47534	2	34.23767	21.98066*
Incubation period	62920.29	5	12584.06	8078.995*
Concentration	6171.21	5	1234.242	792.3863*
Incubation period × Concentration	1620.726	25	64.82905	41.6204*
Error	109.0339	70	1.557627	
Total	70889.73	107	662.5209	

$LSD_{(0.05)}$ 2.034999

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table-6: Effect of different concentrations of cowdung extract on conidial germination of *B. theobromae* after 12 hours of incubation at 24±2°C.

Period of incubation (hours)	Percentage on the germination[1] of conidia in different concentrations (in w/v).						Length of germ tube of conidia (μ)	Correlation (r8)
	1:5	1:10	1:15	1:20	1:25	1:30		
2	0	0	0	0	0	0	0.0	
4	10	20	25	36	10	1	12.85-385.5	-0.3977
6	30	45	47	49	13	2	25.7-449.75	-0.6919
8	34	60	65	68	20	4	25.7-398.35	-0.5832
10	36	75	80	82	25	7	38.55-578.25	-0.5079
12	42	84	100	97	38	10	51.4-α	-0.3964
Correlation (r9)	0.8993	0.9635	0.9802	0.9601	0.9545	0.9943		

1 – Mean of three replications.
Correlation (r8) - Correlation between conidial germination and concentration.
Correlation (r9) - Correlation between conidial germination and incubation period.
α- Immeasurable length of germ tube.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* at different concentration in cowdung extract.

ITEMS	SS	DF	MS	F
Replication	27.35641	2	13.6782	57.38076*
Incubation period	33945.66	5	6789.131	28480.75*
Concentration	20446.17	5	4089.233	17154.54*
Incubation period × Concentration	7289.065	25	291.5626	1223.12*
Error	16.68633	70	0.238376	
Total	61724.93	107	576.8685	

$LSD_{(0.05)}$ 0.796093
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-7: Effect of different types of water solution on conidial germination of *B. theobromae* after eight hours of incubation at 30±2°C.

Different types of water	Percentage of Conidial germination[1]	Length of germ tube of conidia (μ)
Tap water	44	6.42-77.1
Sterilized water	54	6.42-98.96
Pond water	65	6.42-167.05
Distilled water	88	25.7-488.3
Rain water	90	64.25-578.25
River water	93	154.2-449.75

1 – Mean of three replications.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* in different water.

ITEMS	SS	DF	MS	F
Replication	7.3956	2	3.6978	66.92447*
Different water	2955.803	5	591.1607	10699.09*
Error	0.552533	10	0.055253	
Total	2963.751	17	174.3383	

$LSD_{(0.05)}$ 0.427611

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table - 8: Effect of different concentrations of KNO_3 and $NaNO_3$ salt solution on conidial germination of *B. theobromae* after 24 hours of incubation at room temperature (30±2°C).

Name of solution	Period of incubation (hours)	Percentage on the germination[1] of conidia in different concentrations						Length of germ tube of conidia (μ)	Correlation (r10)
		1%	1.5%	2%	2.5%	3%	3.5%		
KNO_3	2	00	00	00	00	00	00	0	-
	4	6	5	3	00	00	00	6.42-64.25	-0.9261
	6	15	12	9	5	00	00	6.42-154.2	-0.9554
	8	25	21	18	11	4	3	6.42-192.75	-0.9784
	10	38	27	25	18	9	7	12.85-288.2	-0.9856
	12	46	37	30	23	15	12	12.85-411.75	-0.9963
	24	48	39	31	24	16	13	51.4-635.25	-0.9964
$NaNO_3$	2	00	00	00	00	00	00	0	-
	4	30	22	15	10	6	04	6.42-64.25	-0.9948
	6	47	41	35	27	18	8	6.42-167.05	-0.9829
	8	62	54	42	35	25	15	6.42-205.6	-0.9973
	10	75	64	53	40	31	24	12.85-321.25	-0.9970
	12	85	72	60	56	38	33	12.85-449.75	-0.9875
	24	88	74	62	57	46	40	64.25-835.25	-0.9804

Correlation (r11) - 0.8331 and 0.7907 for KNO_3 and $NaNO_3$ respectively.

1 – Mean of three replications.

Correlation (r10) - Correlation between conidial germination and concentration of solution.

Correlation (r11) - Correlation between incubation period and conidial germination (average of different concentration).

Analysis of variance on conidial germination of *Botryodiplodia theobromae* in different concentration of KNO_3 solution in different incubation period.

ITEMS	SS	DF	MS	F
Replication	36.93848	2	18.46924	77.90741*
Incubation period	16672.91	6	2778.818	11721.68*
Concentration	5489.589	5	1097.918	4631.264*
Incubation period× Concentration	1189.438	30	39.64793	167.2439*
Error	19.43946	82	0.237067	
Total	23408.31	125	187.2665	

$LSD_{(0.05)}$ 0.792313

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on conidial germination of *Botryodiplodia theobromae* in different concentration of $NaNO_3$ solution in different incubation period.

ITEMS	SS	DF	MS	F
Replication	37.15231	2	18.57616	81.22605*
Incubation period	35878.97	6	5979.828	26147.38*
Concentration	8752.45	5	1750.49	7654.188*
Incubation period× Concentration	1776.336	30	59.21121	258.9068*
Error	18.75316	82	0.228697	
Total	46463.66	125	371.7093	

$LSD_{(0.05)}$ 0.778201

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

4.5 NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING THE GROWTH AND SPORULATION OF *B. theobromae* PAT.

4.5.1 Effect of Media:

To select a suitable culture medium for laboratory studies of *B. theobromae* the following synthetic and non-synthetic liquid and solid media given under materials and methods were tried.

Potato Dextrose Agar (PDA) medium, Potato Agar (PA) medium, Sabouraud's medium, Paper Chromatography medium (PCM), Richard's medium, Czapeck's medium, Cowdung, Fertilizer, Oil-cake (different concentrations) medium and glucose, $NaNO_3$, KNO_3 nitrogen salt are tried in the present study.

The hydrogen-ion concentration of the liquid media was adjusted before autoclaving to 6.5, which was the optimum pH for the growth of *B. theobromae*.

4.5.1.1 Effect of Different Solid Media on Mycelial Growth of *B. theobromae*.

Effect of different solid media on mycelial growth has been studied and results shown in Table-9. Results show that growth of mycelium of *B. theobromae* was the highest (90 mm) both in PDA and cowdung media and the lowest (64mm) in Richard's medium on 4 days of incubation. The growth rate is fairly good in rest of the other tried media.

Range of correlation (r12) values 0.9552 – 0.9955 indicates that there was highly significant relationship between incubation period and mycelial growth. Analysis of variance on mycelial growth of *B. theobromae* in different media shows that the items incubation period, media and incubation period × media were significant. LSD value also indicates significant different of mycelial growth in different media.

4.5.1.2 Effects of Different Concentrations of Solid Cowdung Media on Mycelial Growth.

In the present investigation, effects of different concentrations of solid cowdung media on mycelial growth of *B. theobromae* on 4 days was recorded and shown in Table-10. Results showed that growth of mycelium of *B. theobromae* was the highest (90 mm) in 20% concentration of cowdung and PDA (control) media. 15 and 10% media showed intermediary effect on mycelial growth (74 and 66 mm) of this fungus. In 5% cowdung medium, mycelial growth was lower (34 mm). It is evident from Table-10 that with increase of cowdung in the media, the rate of mycelial growth was increased.

Range of correlation (r13) values 0.9569 – 0.9944 and correlation (r14) values 0.9625 - 0.9926 indicate respectively that there were highly significant relationship between mycelial growth and concentration and incubation period and mycelial growth. Analysis of variance on mycelial growth of *B. theobromae* in different concentration of cowdung medium shows that the items incubation period, concentration and incubation period × concentration were significant. LSD value also indicates significant different of mycelial growth.

4.5.1.3 Effect of Different Media on the Dry Weight of Mycelium and Sporulation of *B. theobromae*.

Dry weight and sporulation of *B. theobromae* was tested after 15th days of incubation and the results are presented in Table-11. It was observed that the highest dry weight (220 mg) was measured in PDA medium and the lowest (110 mg) in Richard's medium. In rest of the tested media (Sabouraud's, PCM and Czapek's), the dry weight of this fungus was measured intermediary (190 mg, 170 mg and 135 mg). The highest sporulation was counted in Richard's medium (96/0.01 ml) and the lowest was in PCM medium (24/0.01 ml). Intermediary sporulation (55/0.01 ml and 70/0.01 ml) was counted in both PDA and Czapek's media. There was no spore found in Sabouraud's medium of the fungus at same period of incubation.

Analysis of variances on dry weight of mycelium and sporulation of *B. theobromae* in different media show that the item media were significant in every case. LSD values also indicate significant different in dry weight of mycelium and sporulation in different media.

4.5.1.4 Effect of Different Concentration of Cowdung Media on the Dry Weight of Mycelium and Sporulation of *B. theobromae* at 28±2°C after 15 days of Incubation.

Dry weight and conidial counts at different concentrations of cowdung and PDA media after 15 days of incubation are presented in Table-12. It was observed that the highest dry weight (330 mg) was measured on 15% cowdung medium and the lowest dry weight (135 mg) was on 5% cowdung medium. It is evident from Table-12 that intermediary types of dry weight (299 mg, 269 mg and 220 mg) was measured on 10% and 20% cowdung and PDA media. The highest (94/0.01 ml) sporulation of the fungus was recorded in 20% cowdung medium. Whereas intermediary types of sporulation (53/0.01 ml, 72/0.01 ml and 57/0.01 ml) was counted on 10% and 15% cowdung and PDA media. There was no spore of the fungus found in 5% cowdung medium.

Analysis of variances on dry weight of mycelium and sporulation of *B. theobromae* in different concentration of cowdung medium show that the item media concentrations were significant in every cases. LSD value also indicate significant different in dry weight of mycelium and sporulation in different media concentrations.

4.5.1.5 Effect of different concentrations of fertilizers media on the mycelial growth and dry weight of *B. theobromae* after four days of incubation.

Effect of different concentrations of fertilizers (urea, gypsum potash, boron, zinc and sulpher) in combination with PA media on the mycelial growth and dry weight *B. theobromae* have been studied and results are presented in Table-13 and

Plate-5. Mycelial growth of *B. theobromae* was measured the highest in boron fertilizer medium (90 mm) than that of other tested media after 4 days of incubation. Results showed that growth of mycelium of *B. theobromae* was the highest (90 mm) in 3% concentration of boron fertilizer and the lowest (45mm) in 3% concentration of potash fertilizer media after 4 days of incubation. On the other hand, except sulpher containing media, rest of the tested media had intermediary effect on mycelial growth of *B. theobromae*. It is evident from the study that increase of fertilizer in the media, the growth of mycelium of the fungus was increased. In case of sulpher containing media, the highest mycelial growth (65 mm) was observed in 2% medium. The rate of mycelial growth was decreased with the increase or decrease of sulpher concentrations in the medium. Results show that the dry weight of mycelia was the highest (240 mg) in 3% concentration of boron containing medium and the lowest (50 mg) in 3% concentration of urea and gypsum media.

Ranges of correlation (r15) values 0.9665 – 0.9992, 0.9829 – 0.9979, 0.9762 – 0.9926, 0.8226 – 0.9387, 0.9613 – 0.9976 and 0.9825 – 0.9994 for urea, gypsum, potash, boron, zinc and sulpher respectively indicate that there were highly significant relationship between incubation period and radial growth of mycelium. Correlation (r16) values -0.9958, -0.9689, -0.9395, 0.9729, 0.9570 and -0.6776 for urea, gypsum, potash, boron, zinc and sulpher respectively indicate that relationship between concentration and radial growth of mycelium are highly negative significant in case of urea, gypsum, potash and sulpher highly significant in case of boron and zinc. Analysis of variance on radial mycelial growth of *B. theobromae* in different concentrations (1–3%) of urea, gypsum, potash, boron, zinc and sulpher show that the items concentration, incubation period and concentration × incubation period are significant in every case. LSD value also indicate significant different in radial growth of mycelium in every case.

4.5.1.6 Effect of different concentrations of mustard and linseed oil-cake media on the mycelial growth and dry weight of *B. theobromae* after four days of incubation at (28 ± 2) °C.

Effect of different concentrations of mustard and linseed oil-cake on the mycelial growth of *B. theobromae* has been studied and results are shown in Table-14 and **Plate-6A**. Result shows that the mycelial growth of *B. theobromae* was increased with the increase of concentrations of both mustard and linseed oil-cake media. The highest mycelial growth (90 mm) was measured in 10, 15 and 20% concentrations of mustard oil-cake medium and the lowest (80 mm) in 5% concentration of mustard oil-cake medium after 4 days of incubation. On the other hand, the highest mycelial growth of *B. theobromae* was measured (90 mm) in 15 and 20% concentrations and the lowest (67 mm) in 5% concentration of linseed oil cake. Mycelial growth of the fungus was recorded 80, 90, 90 and 90 mm in 5, 10, 15 and 20% concentrations of mustard oil-cake medium. Whereas 67, 78, 90 and 90 mm of mycelial growth were found in 5, 10, 15 and 20% concentrations of linseed oil-cake medium. It is evident from the experiment that mustard oil-cake is more favorable for the growth of *B. theobromae* than that of linseed oil-cake. The highest dry weight of mycelia (340 mg) was measured in 20% concentration and the lowest (170 mg) at 5% concentration of mustard oil-cake medium.

Ranges of correlation (r17) values 0.9891 – 0.9990 and 0.9899 – 0.9984 for mustard oil-cake and linseed oil-cake respectively indicate that there were highly significant relationship between incubation period and radial growth of mycelium. Correlation (r18) values 0.9923 and 0.9790 for mustard oil-cake and linseed oil-cake respectively also indicate highly significant relationship between concentration (5–20%) and radial growth of mycelium. Analysis of variance on radial mycelial growth of *B. theobromae* in different concentration (5–20%) of mustard oil-cake and linseed oil-cake show that the items concentration, incubation period and concentration × incubation period are significant in every case. LSD value also indicate significant different in radial growth of mycelium in every case.

4.5.1.7 Effect of Different Concentrations of KNO_3 and $NaNO_3$ in Combination with PA Medium on Mycelial Growth and Dry Weight of *B. theobromae* after seven days of Incubation at 28±2°C.

Effect of different concentrations of KNO_3 and $NaNO_3$ incombination with potato-agar (PA) media was tested for mycelial growth and dry weight of *B. theobromae* after 7 days of incubation and the results are presented in Table-15. It is observed from Table-15 that in PA containing KNO_3 media, mycelial growth and dry weight was faster only (82 mm and 120 mg) and slower on 3.5% KNO_3 containing PA medium (39 mm and 10 mg **Plate 6B**). Mycelial growth rate and dry weight were gradually decreased (82, 78, 75, 68, 66, 62, 53 and 39 mm and 120, 100, 60, 60, 40, 30, 20 and 10 mg) with the increase of KNO_3 (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5%) in the media. On the other hand, $NaNO_3$ containing PA media, the highest mycelial growth and dry weight were observed only on PA medium (80 mm and 110mg) and the lowest on 3.0% $NaNO_3$ containing PA medium (08 mm and 20 mg **Plate 6C**). Growth and dry weight of this fungus gradually decreased (80, 51, 49, 42, 35, 20 and 08 mm and 110, 70, 70, 50, 50, 40 and 20 mg) with the increase of $NaNO_3$ concentrations (0, 0.5, 1.5, 1.5, 2.0,2.5 and 3.0 %). No mycelial growth was found on 3.5% of $NaNO_3$ containing PA medium.

Correlation (r19) values –0.9659 and -0.9778 for mycelial growth in KNO_3 and $NaNO_3$ and -0.9684 and –0.9643 for dry weight of mycelium in KNO_3 and $NaNO_3$ indicate that there was highly negative significant reationship between concentration and mycelial growth and dry weight of mycelium. Analysis of variances on mycelial growth and dry weight of mycelium of *B. theobromae* in different concentration of KNO_3 and $NaNO_3$ in PA shows that the items treatment, salt and treatment × salt were significant. LSD value also indicates significant different in mycelial growth and dry weight of mycelium in every cases.

4.5.1.8 Effect of Leaf Extracts Media of some Plants on the Growth of *B. theobromae* on 7 days in Incubation.

The effect of different plant extracts considered as media on mycelial growth and dry weight of *B. theobromae* was tested and the results are presented in Table 16. The highest (90 mm) mycelial growth was found in *Euphorbia hirta* (on 4th day after incubation) and the lowest (20 mm) in *Datura metel* and *Polygonum orientale*. The mycelial growth of the fungus showed intermediary on *Lantana camara*, *Cucurbita* sp, *Tagetes patula* media and rest of the other tested media had moderate effect (**Plate-7**). The highest (200 mg) dry weight of mycelium was measured on *Euphorbia hirta* and the lowest (10 mg) on *Datura metel* and *Clerodendrun viscosum* medium, in rest of the tested media (*Ocimum sanctum*, *Vinca rosea*, *Adhatoda vasica*, *Rauvolfia serpentina*, *Zizyphus jujuba*) the dry weight of this fungus was intermediary (170,160,150,120 and 110mg), and other tested media had moderate effect.

Range of correlation (r20) values 0.8998 – 0.9975 indicates that there was highly significant relationship between mycelial growth and incubation period. Analysis of variance on mycelial growth of *B. theobromae* in different plant extracts media show that the item extracts, incubation period and extracts × incubation period were significant. LSD value also indicates significant different in mycelial growth.

PLATE -5

Photograph showing mycelial growth of *B. theobromae* in different fertilizers media.

1. A_1 - 1%, A_2 - 2% and A_3 - 3%, Urea media
2. B_1 - 1%, B_2 - 2% and B_3 - 3%, Gypsum media
3. C_1 - 1%, C_2 - 2% and C_3 - 3%, Potash media
4. D_1 - 1%, D_2 - 2% and D_3 - 3%, Boron media
5. E_1 - 1%, E_2 - 2% and E_3 - 3%, Zinc media
6. F_1 - 1%, F_2 - 2% and F_3 - 3%, Sulpher media

PLATE - 5

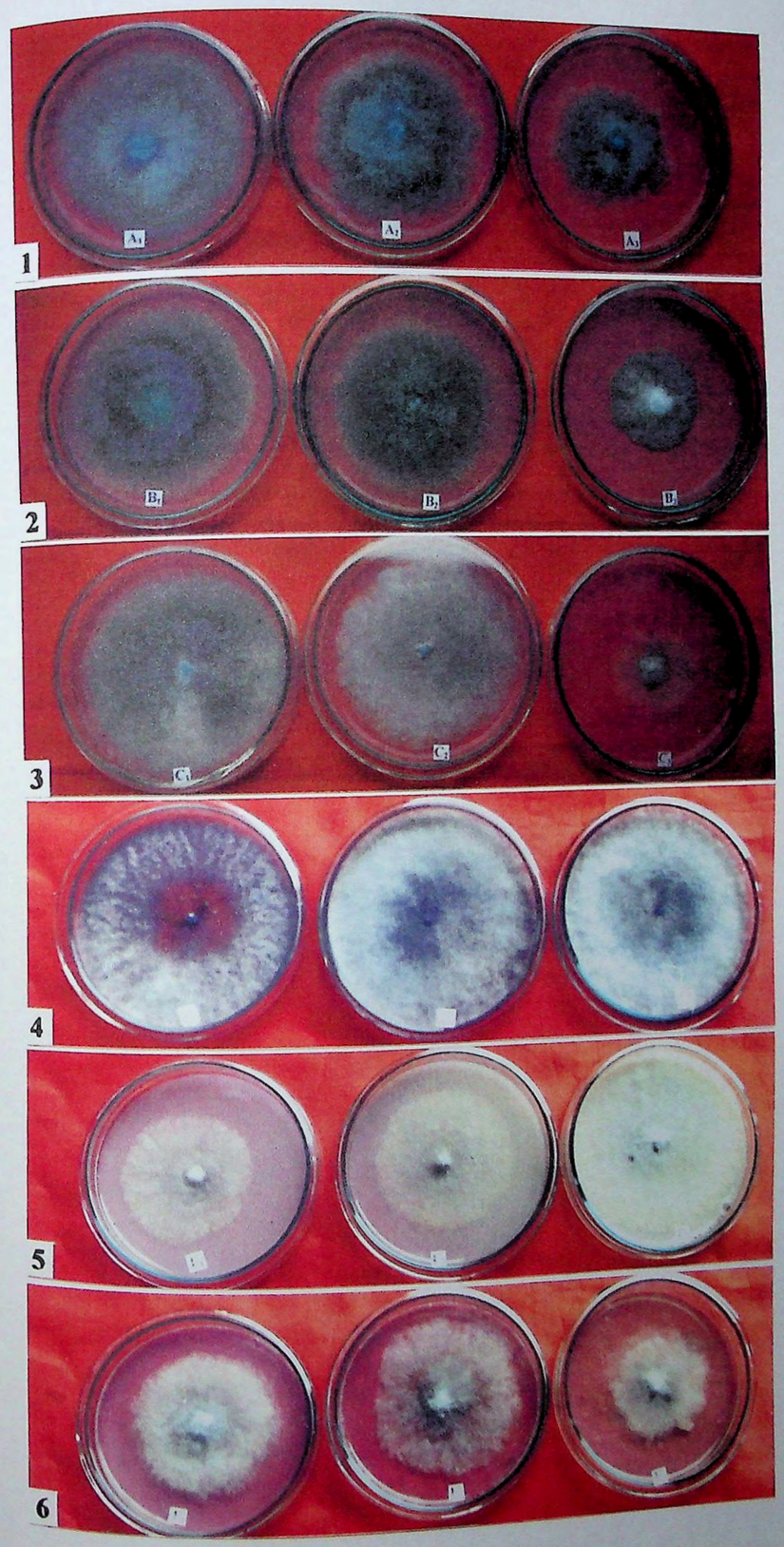


PLATE - 6

A. Photograph showing mycelial growth of *B. theobromae* in different percentages of mustard and linseed oil-cake media.
A_1 - 5%, A_2 -10%, A_3 - 15% and A_4 - 20%
mustard oil-cake media
B_1 - 5%, B_2 -10%, B_3 - 15% and B_4 - 20%
linseed oil-cake media

B. Photograph showing mycelial growth of *B. theobromae* in different percentages of KNO_3 salt media.
(1) PA (2) .5% (3) 1% (4) 1.5%
(5) 2% (6) 2.5% (7) 3% (8) 3.5%

C. Photograph showing mycelial growth of *B. theobromae* in different percentages of $NaNO_3$ salt media.
(1) PA (2) .5% (3) 1% (4) 1.5%
(5) 2% (6) 2.5% (7) 3% (8) 3.5%

PLATE - 6

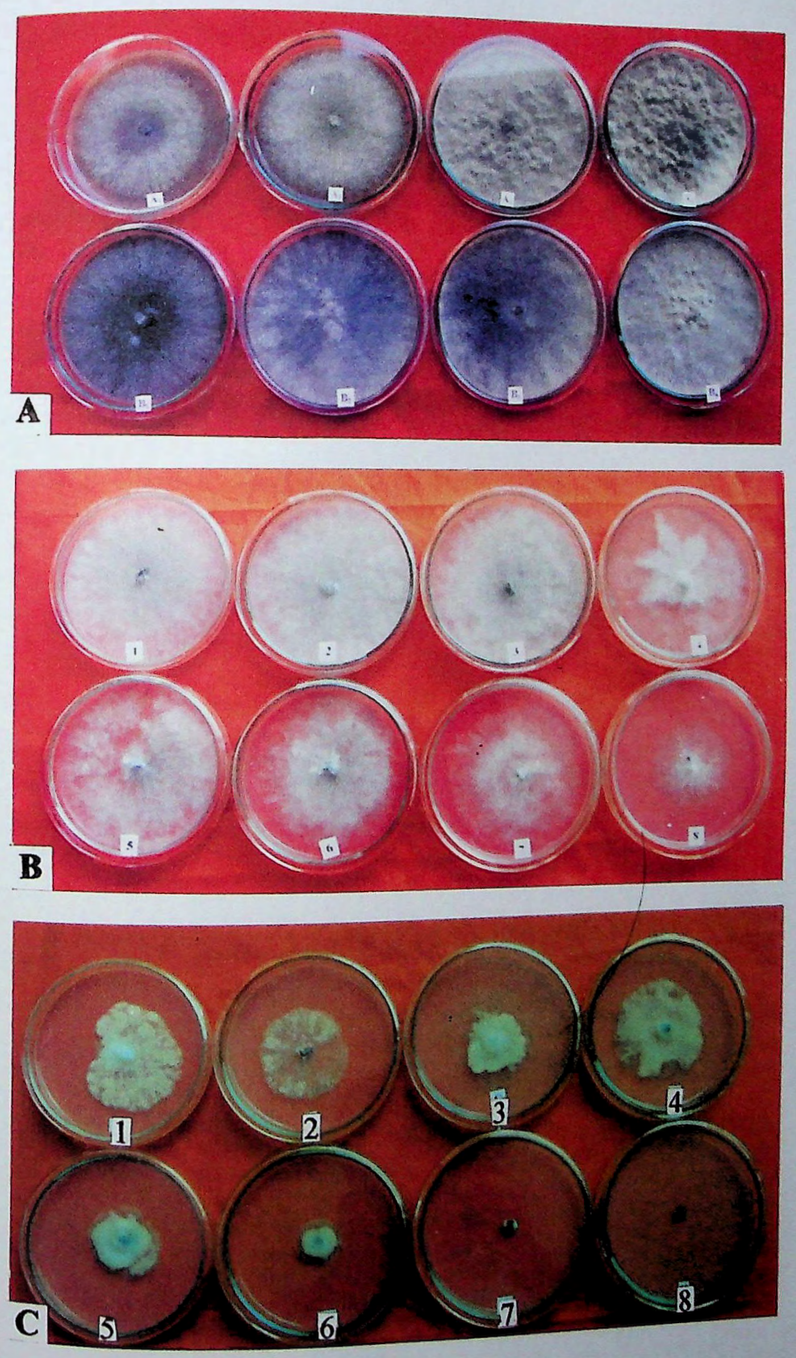


PLATE -7

Photograph showing mycelial growth of *B. theobromae* in different plant-extracts media.

1. *Moringa olifera*
2. *Acalypha indica*
3. *Clerodendrum viscosum*
4. *Cajanus sp.*
5. *Curcuma longa*
6. *Polygonum orientale*
7. *Datura metel*
8. *Cucurbita sp.*
9. *Solanum melongena*
10. *Lantana camara*
11. *Ocimum sanctum*
12. *Euphorbia hirta*
13. PDA (control)

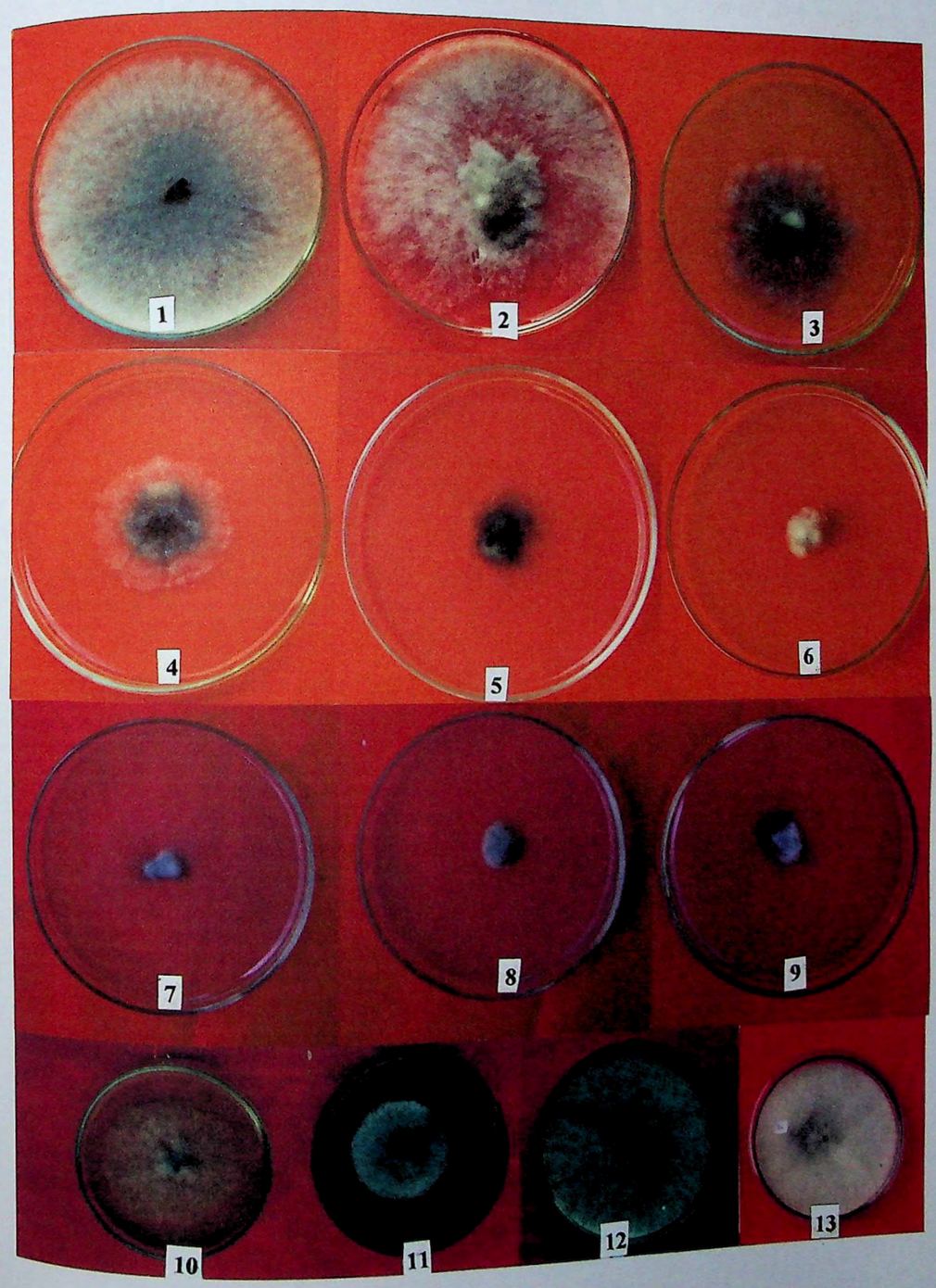


Table-9: Effect of different solid media on the mycelial growth of *B. theobromae* at 28±2°C after four days of incubation.

Period of incubation (days)	Radial growth of mycelium[1] in mm after the incubation in different media					
	PDA	PCM	Sabouraud's	Czapek's	Richard's	Cowdung
1	10	07	05	05	03	08
2	30	25	22	20	18	32
3	85	78	62	53	41	74
4	90	88	85	72	64	90
Correlation (r12)	0.9552	0.9653	0.9888	0.9901	0.9955	0.9867

1 – Mean of three replications.
Correlation (r12) - Correlation between incubation period and mycelial growth.

Analysis of variance on mycelial growth of *Botryodiplodia theobromae* in different media at different incubation period.

ITEMS	SS	DF	MS	F
Replication	65.33333	2	32.66667	102.4545*
Incubation period	66000.38	3	22000.13	69000.39*
Media	4461.625	5	892.325	2798.656*
Incubation period× Media	2017.875	15	134.525	421.9193*
Error	14.66667	46	0.318841	
Total	72559.88	71	1021.97	

$LSD_{(0.05)}$ 0.928863
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-10: Effect of different concentrations of cowdung extract medium on the mycelial growth of *B. theobromae* at 28±2°C after four days of incubation.

Period of incubation in days	Radial growth of mycelium in mm[1] after incubation in different concentrations				Correlation (r13)
	20%	15%	10%	5%	
1	07	05	03	00	0.9944
2	25	20	17	05	0.9569
3	70	48	38	15	0.9899
4	90	74	66	34	0.9648
Correlation (r14) -	0.9846	0.9926	0.9891	0.9625	

1 – Mean of three replications.
Correlation (r13) – Correlation between mycelial growth and concentration.
Correlation (r14) - Correlation between incubation period and mycelial growth (average of different concentration)

Analysis of variance on mycelial growth of *Botryodiplodia theobromae* at different concentration of cowdung extract media in different incubation period.

ITEMS	SS	DF	MS	F
Replication	28.9	2	14.45	107.6667*
Incubation period	39953.4	3	13317.8	99230.67*
Concentration	12077.1	4	3019.275	22496.56*
Incubation period × Concentration	4652.1	12	387.675	2888.559*
Error	5.1	38	0.134211	
Total	56716.6	59	961.2983	

$LSD_{(0.05)}$ 0.605781
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table 11: Effect of different media on the dry weight of mycelium and sporulation of *B. theobromae* at 28±2°C after 15 days of incubation.

Name of media	Dry weight of mycelium in mg[1]	Sporulation (Conidia/0.01ml)
PDA	220	55
PCM	170	24
Sabouraud's	190	00
Czapek's	135	70
Richard's	110	96

1 – Mean of three replications.

Analysis of variance on mycelial dry weight of *Botryodiplodia theobromae* in different media.

ITEMS	SS	DF	MS	F
Replication	32.4	2	16.2	81*
Media	22800	4	5700	28500*
Error	1.6	8	0.2	
Total	22834	14	1631	

$LSD_{(0.05)}$ 0.842032

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on sporulation of *Botryodiplodia theobromae* in different media.

ITEMS	SS	DF	MS	F
Replication	14.4	2	7.2	10.28571*
Media	17136	4	4284	6120*
Error	5.6	8	0.7	
Total	17156	14	1225.429	

$LSD_{(0.05)}$ 1.575298

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table 12: Effect of different concentrations of cowdung extract and other solid media on the dry weight of mycelium and sporulation of *B. theobromae* at 28±2°C after 15 days of incubation

Media	Dry weight of mycelium in mg[1]	Sporulation (Conidia/0.01ml)
20%	269	94
15%	330	72
10%	299	53
5%	135	00
PDA (control)	220	57

1 – Mean of three replications.

Analysis of variance on mycelial dry weight of *Botryodiplodia theobromae* in different concentration of cowdung extract media.

ITEMS	SS	DF	MS	F
Replication	24.5	2	12.25	49*
Media concentration	66344.25	3	22114.75	88459*
Error	1.5	6	0.25	
Total	66370.25	11	6033.659	

$LSD_{(0.05)}$ 0.998984

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on sporulation of *Botryodiplodia theobromae* in different concentration of cowdung extract media.

ITEMS	SS	DF	MS	F
Replication	4.5	2	2.25	9*
Media concentration	14516.25	3	4838.75	19355*
Error	1.5	6	0.25	
Total	14522.25	11	1320.205	

$LSD_{(0.05)}$ 0.998984

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table -13: Effect of different concentrations of fertilizers (urea, gypsum potash, boron, zinc and sulpher) media on the mycelial growth and dry weight of *B. theobromae* after four days of incubation.

Name of the Fertilizers	Concentrations (%)	Radial growth of mycelium[1] (mm) on different incubation period (days)				Correlation (r15)	Dry weight of mycelia (mg)
		1	2	3	4		
Urea	1	16	40	84	90	0.9665	90
	2	14	35	57	82	0.9992	70
	3	10	25	39	56	0.9992	50
Gypsum	1	12	30	55	78	0.9979	90
	2	10	28	50	75	0.9974	80
	3	08	20	38	66	0.9829	50
Potash	1	20	35	63	90	0.9920	200
	2	15	30	58	84	0.9926	160
	3	08	15	26	45	0.9762	80
Boron	1	20	58	90	90	0.9387	150
	2	24	78	90	90	0.8603	170
	3	28	84	90	90	0.8226	240
Zinc	1	00	08	21	49	0.9613	60
	2	00	10	29	58	0.9766	70
	3	00	22	54	82	0.9976	190
Sulpher	1	00	18	38	62	0.9978	110
	2	00	20	44	65	0.9994	150
	3	00	09	30	54	0.9825	140

Correlation (r16) - -0.9958, -0.9689, -0.9395, 0.9729, 0.95700 and -0.6776 for Urea, Gypsum, Potash, Boron, Zinc and Sulpher respectively.

1 - Mean of three replication.

Correlation (r15) – Correlation between incubation period and radial growth of mycelium.

Correlation (r16) - Correlation between concentration and radial growth of mycelium.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of urea

ITEMS	SS	DF	MS	F
Replication	73.5	2	36.75	49*
Concentration	3782	2	1891	2521.333*
Incubation period	20908	3	6969.333	9292.444*
Concentration × Incubation period	1598	6	266.3333	355.1111*
Error	16.5	22	0.75	
Total	26378	35	753.6571	

$LSD_{(0.05)}$ 1.466539

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of gypsum.

ITEMS	SS	DF	MS	F
Replication	40.05556	2	20.02778	66.64706*
Concentration	726.2222	2	363.1111	1208.336*
Incubation period	20110.75	3	6703.583	22307.72*
Concentration × Incubation period	154	6	25.66667	85.41176*
Error	6.611111	22	0.300505	
Total	21037.64	35	601.0754	

$LSD_{(0.05)}$ 0.928301

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of potash.

ITEMS	SS	DF	MS	F
Replication	84.38889	2	42.19444	50.78723*
Concentration	5533.556	2	2766.778	3330.225*
Incubation period	18103.33	3	6034.444	7263.343*
Concentration × Incubation period	1356.667	6	226.1111	272.1581*
Error	18.27778	22	0.830808	
Total	25096.22	35	717.0349	

$LSD_{(0.05)}$ 1.543524

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of boron.

ITEMS	SS	DF	MS	F
Replication	32.66667	2	16.33333	10.16981*
Concentration	458	2	229	142.5849*
Incubation period	26328	3	8776	5464.302*
Concentration × Incubation period	750	6	125	77.83019*
Error	35.33333	22	1.606061	
Total	27604	35	788.6857	

$LSD_{(0.05)}$ 2.146071

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of zinc.

ITEMS	SS	DF	MS	F
Replication	32.66667	2	16.33333	23.43478*
Concentration	2620.5	2	1310.25	1879.924*
Incubation period	20414.75	3	6804.917	9763.576*
Concentration × Incubation period	1247.5	6	207.9167	298.3152*
Error	15.33333	22	0.69697	
Total	24330.75	35	695.1643	

$LSD_{(0.05)}$ 1.413742

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of sulpher.

ITEMS	SS	DF	MS	F
Replication	20.16667	2	10.08333	22.55932*
Concentration	510.5	2	255.25	571.0678*
Incubation period	18614	3	6204.667	13881.63*
Concentration × Incubation period	185.5	6	30.91667	69.16949*
Error	9.833333	22	0.44697	
Total	19340	35	552.5714	

$LSD_{(0.05)}$ 1.132145

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table -14: Effect of different concentrations of mustard and linseed oil-cake media on the mycelial growth and dry weight of *B. theobromae* after four days of incubation at (28 ± 2) °C.

Name of the oil-cake	Concentrations (%)	Radial growth of mycelium[1] (mm) on different incubation period (days)				Correlation (r17)	Dry weight of mycelium (mg)
		1	2	3	4		
Mustard oil-cake	5	06	30	58	80	0.9990	170
	10	10	33	65	90	0.9981	240
	15	14	37	72	90	0.9931	290
	20	17	45	76	90	0.9891	340
Linseed oil-cake	5	04	23	48	67	0.9984	190
	10	11	28	59	78	0.9936	210
	15	16	34	71	90	0.9899	280
	20	18	40	74	90	0.9915	320

Correlation (r18) - 0.992333 and 0.979057 for Mustard oil-cake and Linseed oil-cake respectively.
1 - Mean of three replication.
Correlation (r17) – Correlation between incubation period and radial growth of mycelium.
Correlation (r18) - Correlation between concentrations and radial growth of mycelium.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of Mustard oil-cake.

ITEMS	SS	DF	MS	F
Replication	34.04167	2	17.02083	40.44554*
Concentration	1190.5	3	396.8333	942.9703*
Incubation period	40483.5	3	13494.5	32066.14*
Concentration × Incubation period	182	9	20.22222	48.05281*
Error	12.625	30	0.420833	
Total	41902.67	47	891.5461	

$LSD_{(0.05)}$ 1.081596
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on radial growth of mycelium of *B. theobromae* in different concentration of Linseed oil-cake.

ITEMS	SS	DF	MS	F
Replication	40.5	2	20.25	52.82609*
Concentration	2958.563	3	986.1875	2572.663*
Incubation period	34616.06	3	11538.69	30100.92*
Concentration × Incubation period	258.1875	9	28.6875	74.83696*
Error	11.5	30	0.383333	
Total	37884.81	47	806.0598	

$LSD_{(0.05)}$ 1.032282
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table 15: Effect of Defferent concentration of KNO_3 and $NaNO_3$ salt in combination with PA medium on mycelial growth and dry weight of *B. theobromae* after seven days in incubation at 28±2°C.

Treatment	Mycelial growth[1] in (mm)		Dry weight of mycelium[1] (mg)	
	KNO_3	$NaNO_3$	KNO_3	$NaNO_3$
PA	82	80	120	110
0.5% +PA	78	51	100	70
1.0% +PA	75	49	60	70
1.5% +PA	68	42	60	50
2.0% +PA	66	35	40	50
2.5% +PA	62	20	30	40
3.0% +PA	52	08	20	20
3.5% +PA	39	00	10	00
Correlation (r19)	-0.9659	-0.9778	-0.9684	-0.9643

1 – Mean of three replications.

Correlation (r19) – Correlation between salts concentration and mycelial growth and dry weight of mycelium.

Analysis of variance on mycelial growth of *Botryodiplodia theobromae* in different concentration of KNO_3 and $NaNO_3$ in media.

ITEMS	SS	DF	MS	F
Replication	32	2	16	120*
Treatment	16501.31	7	2357.33	17679.98*
Salt	10531.69	1	10531.69	78987.66*
Treatment× Salt	1868.813	7	266.9732	2002.299*
Error	4	30	0.133333	
Total	28937.81	47	615.6981	

$LSD_{(0.05)}$ 0.608807

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on dry weight of mycelium of *Botryodiplodia theobromae* in different concentration of KNO_3 and $NaNO_3$ in media.

ITEMS	SS	DF	MS	F
Replication	699.2917	2	349.6458	1.633509*
Treatment	41864.58	7	5980.655	27.941*
Salt	752.0833	1	752.0833	3.513656*
Treatment× Salt	4164.583	7	594.9405	2.7795*
Error	6421.375	30	214.0458	
Total	53901.92	47	1146.849	

$LSD_{(0.05)}$ 24.3929

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table 16: Effect of Leaf Extracts Media of Some plants on mycelial growth and dry weight of *B. theobromae* at 28±2°C.

Source of leaf extract	Radial growth of mycelium[1] (mm) on different leaf extracts media after incubation period (days)							Dry weight of mycelium (mg)	Correlation (r20)
	1	2	3	4	5	6	7		
Euphorbia hirta	8	36	54	90	90	90	90	200	0.8998
Ocimum sanctum	6	13	20	28	32	38	45	170	0.9975
Lantana camara	0	11	18	36	45	52	65	60	0.9950
Datura metel	0	0	4	8	12	18	20	10	0.9841
Solanum melongena	0	0	6	10	15	20	25	80	0.9895
Cucurbita sp.	0	0	6	20	35	50	65	70	0.9729
Polygonum orientale	0	0	3	10	12	15	20	20	0.9810
Tagetes patula	0	0	5	28	51	70	85	80	0.9705
Vinca rosea	2	8	18	37	58	75	90	160	0.9893
Curcuma longa	0	4	7	9	12	18	28	40	0.9615
Cajanus sp.	0	0	5	12	25	33	40	30	0.9752
Clerodendrum viscosum	0	0	4	11	23	36	50	10	0.9569
Moringa olifera	0	0	5	26	51	70	90	160	0.9674
Acalypha indica	0	3	15	31	49	71	90	90	0.9713
Rauvolfia serpentina	1	4	17	34	52	75	90	120	0.9859
Zizyphus jujuba	2	6	19	36	53	76	90	110	0.9878
Adhatoda vasica	2	5	18	33	54	73	90	150	0.9859
PDA (control)	5	19	42	69	90	90	90	240	0.9535

1 – Mean of three replications.
Correlation (r20) – Correlation between mycelial growth and incubation period.

Analysis of variance on radial growth of mycelium of *Botryodiplodia theobromae* in different plant extracts media media.

ITEMS	SS	DF	MS	F
Replication	134.3492	2	67.1746	338.2353*
Extracts	94936.19	17	5584.482	28118.79*
Incubation period	190776	6	31795.99	160098.1*
Extracts × Incubation period	47097.48	102	461.74	2324.937*
Error	49.65079	250	0.198603	
Total	332993.6	377	883.2722	

$LSD_{(0.05)}$ 0.713187
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

4.6 EFFECT OF TEMPERATURE AND LIGHT ON THE GROWTH AND SPORULATION OF *Botryodiplodia theobromae.*

4.6.1 Effect of Temperature on Mycelial Growth and Sporulation.

The mycelial growth and conidial counts at different temperatures after three and fifteen days of incubation are presented in Table-17. It was observed that the temperatures range of 25°-30°C was optimum for mycelial growth of *B. theobromae* on PDA (78-90 mm) and cowdung (80-90 mm) media. But rest of the temperatures had intermediary effect on mycelial growth of this fungus. The relative abundance of the mycelia increased with increase of temperatures. In both the media, growth and sporulation of the fungus was found to be inhibited at 45°C. There was no mycelial growth and sporulation at temperature 10° and 45°C. Sporulation occurred at between 15°- 40°C. In both media, the highest sporulation occurred at 30°C (38 conidia/0.01 ml and 42 conidia/0.01 ml) and the lowest was at 15°C (5 conidia/ 0.01 ml and 7 conidia/0.01 ml).

Correlation (r21) values 0.0554 and 0.0773 for mycelial growth in PDA and cowdung media and 0.1427 and 0.0436 for sporulation in PDA and cowdung media indicate that there was a low significant reationship between temperature and mycelial growth and sporulation. Analysis of variances on mycelial growth and sporulation of *B. theobromae* in PDA and cowdung media shows that the items temparature, media and temparature × media were significant. LSD value also indicates significant different in mycelial growth and sporulation in every cases.

4.6.2 Effect of Light on the Mycelial Growth of *B. theobromae.*

Botryodiplodia theobromae was inocubated under different light exposures for the observation of growth and sporulation after four and fifteen days on PDA (Table-18). The growth of *B. theobromae* was affected much by different light conditions on PDA. *B. theobromae* sporulated in all the light conditions and the growth and sporulation occurred, while it was the highest in continuous light (90 mm and 106 conidia/ 0.01 ml). The lowest sporulation occurred in continuous

darkness (22 conidia/ 0.01 ml) and by that time growth was measured to be 76 mm. It is evident from Table-18 that sporulation was fairly good (100 conidia/0.01 ml) in alternating light and darkness 12 hours and growth was measured to be 50 mm after fifteen and four days of incubation. Rest of the light conditions viz., 16 hours light 8 hours darkness, 8 hours light 16 hours darkness, alternating light and darkness (24 hours) (45 mm and 44 conidia/0.01 ml, 38 mm and 65 conidia/0.01 ml and 42 mm and 92 conidia/0.01 ml respectively) has intermediary effect in comparison with room conditions (85 mm and 69 conidia/0.01 ml).

Range of correlation (r22) values 0.9622 – 0.9997 indicates that there was highly significant relationship between mycelial growth and incubation period. Analysis of variance on mycelial growth of *B. theobromae* in different light condition shows that the items light condition, incubation period and light condition × incubation period were significant. LSD value also indicates significant different in mycelial growth.

4.6.3 Effect of storage under laboratory conditions on the viability of conidia of *B. theobromae*.

Conidial viability of *B. theobromae* was tested under laboratory condition and results are presented in Table-19. The viability of *B. theobromae* was remained 100% up to 2 months of storage at room temperature (28±2) °C. It is evident from the experiment that after 2 months of storage, the viability of conidia began to lose. 97-54% viability of conidia remained from 3-8 month of storage. After 10 months of storage, conidia of *B. theobromae* showed 38% viability and the experiment was not further continued.

Correlation (r23) value 0.9933 indicates that there was a highly negative significant relationship between month (1 - 10) of storage and percentage of viable conidia. Analysis of variance on percentage viable conidia of *B. theobromae* in different storage month show that the items month is significant at 5% level of probability. LSD value also indicates same result.

Table-17: Effect of temperatures on the growth and sporulation of *B. theobromae* on PDA and cowdung (20% concentration) media recorded after three and fifteen days of incubation.

Temperature (°C)	Mycelial growth[1] (mm)		Sporulation[1] (conidia/0.01ml)	
	PDA	Cowdung	PDA	Cowdung
10	0	0	0	0
15	12	15	5	7
20	58	62	21	25
25	78	80	27	31
30	90	90	38	42
35	71	75	23	24
40	16	25	15	10
45	0	0	0	0
Correlation (r21)	0.0554	0.0773	0.1427	0.0436

1 – Mean of three replications.
Correlation (r21) – Correlation between temperature and mycelial growth, temperature and sporulation.

Analysis of variance on radial growth of mycelium of *Botryodiplodia theobromae* in different temperature and media.

ITEMS	SS	DF	MS	F
Replication	10.125	2	5.0625	19.28571*
Temperature	58563	7	8366.143	31871.02*
Media	90.75	1	90.75	345.7143*
Temperature× Media	98.25	7	14.03571	53.46939*
Error	7.875	30	0.2625	
Total	58770	47	1250.426	

$LSD_{(0.05)}$ 0.85423
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Analysis of variance on sporulation of *Botryodiplodia theobromae* in different temperature and media.

ITEMS	SS	DF	MS	F
Replication	24.5	2	12.25	31.95652*
Temperature	8820	7	1260	3286.957*
Media	18.75	1	18.75	48.91304*
Temperature× Media	98.25	7	14.03571	36.61491*
Error	11.5	30	0.383333	
Total	8973	47	190.9149	

$LSD_{(0.05)}$ 1.032282
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-18: Effect of light on mycelial growth and sporulation of *B. theobromae* after four and fifteen days on PDA medium.

Light condition	Mycelial growth in (mm) different days after incubation.				Sporulation (conidia/ 0.01ml)	Correlation (r22)
	1	2	3	4		
Continuous light	12	40	69	90	106	0.9977
16 hours light 8 hours darkness	5	14	22	45	44	0.9646
8 hours light 16 hours darkness	4	12	20	38	65	0.9761
Alternating light and darkness (12 hours)	7	16	27	50	100	0.9735
Alternating light and darkness (24 hours)	5	11	21	42	92	0.9622
Continuous darkness	8	32	53	76	22	0.9997
Room condition 30±2°C	10	28	62	85	67	0.9935

1 – Mean of three replications

Correlation (r22) - Correlation between mycelial growth and incubation period.

Analysis of variance on radial growth of mycelium of *Botryodiplodia theobromae* in different light condition.

ITEMS	SS	DF	MS	F
Replication	77.78571	2	38.89286	171.9474*
Light condition	14756.14	6	2459.357	18072.95*
Incubation period	33539.14	3	11179.71	49426.11*
Light condition × Incubation period	4695.957	18	260.881	1153.368*
Error	12.21429	54	0.22619	
Total	53081.14	83	639.5318	

$LSD_{(0.05)}$ 0.77909

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table -19: Effect of storage under laboratory conditions on the viability of conidia of *B. theobromae*.

Germination[1] test after months of storage	Percentage of viable conidia
01	100
02	100
03	97
04	92
05	86
06	78
07	60
08	54
09	46
10	38
Correlation (r23)	-0.9933

1 – Mean of three replications.
Correlation (r23) – Correlation be tween storage month and percentage of viable conidia.

Analysis of variance on viability of *B. theobromae* in different month.

ITEMS	SS	DF	MS	F
Replication	20.60766	2	10.30383	8.605939*
Month	9948.142	9	1105.349	923.2069*
Error	21.55127	18	1.197293	
Total	9990.301	29	344.4931	

$LSD_{(0.05)}$ 1.877071
ns= non significant at 5% level of probability.
*= Significant at 5% level of probability.

4.7 CONTROL MEASURES

Some fungicides (different groups), some plant extracts and smokes were used in the present study for the control of *B. theobromae*. The experiments were set up *in vitro* to find out the inhibition of this fungus in fungicides, plant extracts and smoke. Plant extracts have no such hazard role in nature, which will not harmful for human and other animals.

4.7.1. Effect of Fungicides on *B. theobromae in vitro.*

Evaluation of fungicides in laboratory on the inactivation of the pathogen is shown in Table-20. In order to find out the most effective and suitable fungicides for the control of *B. theobromae* following fungicides were evaluated in the laboratory by the technique suggested under materials and methods.

COPPER

FUNGICIDES

Cupravit : (Copper oxychloride).

SULPHUR FUNGICIDES

Thiovit : (Poly sulphide).

CARBAMATE

Dithane M-45 : (Manganous ethlene bisdithiocarbamate + zinc sulphate).

Bavistin : (Methyl-2-benzimidazole carbamate)

Redomil MZ : Methyle-D, L-P (2,4-Dimethylphenyl)-N (2-Methobis acetyl, aianinite); Zinc salt and polymeric manganese ethylene bis (Dithiocarbamate).

Benomyl :

Rovral :

The fungicides were tested in several concentrations. The threads containing fungus were immersed in the fungicides solutions and allowed to remain for different solution and different time period. After removing from the fungicidal solution, they were then placed separately on a piece of sterilized filter paper just to remove excess of solution and planted individual in 2% agar plates. The experiment was done in triplicate. The absence of the fungal growth was taken as indication of effectiveness of the fungicides. The threads along with the fungus are immersed only in sterile water, for different time period instead of fungicidal solution, were put in agar plates for control.

Out of the seven fungicides tested, Cupravit, Dithane-M-45, Benomyl and Redomil-MZ were the most effective against *B. theobromae*, when the fungus was immersed for 5-30 minutes at 0.05–0.25% concentrations. Hundrad per cent (100%) conidial germination inhibition occurred after treating with the four fungicides (Cupravit, Dithane M-45, Benomyl and Redomil-MZ) in all cases of immersion period and concentrations. Rest of the three fungicides (Thiovit, Rovral and Bavistin) has moderate to good inhibitory effect (34, 79 and 88%) against *B. theobromae* at the concentration of 0.05 after an immersion period of 30 minutes. The 0.25% concentration has fairly good effect on the inhibition of the fungus with the application of this three fungicides (Theovit, Rovral and Bavistin). With the increase of concentrations and immersion period, the inhibition of conidial germination of the fungus was also increased.

Range of correlation (r24) values 0.9339 - 0.9938, 0.9126 - 0.9895 and 0.8375 - 0.9645 for Thiovit, Rovral and Bavistin respectively indicate that there was highly significant reationship between conidial germination inhibition and immersion period. Correlation (r25) values 0.9750, 0.9936 and 0.9646 for Thiovit, Rovral and Bavistin respectively indicate that there was highly significant reationship between conidial germination inhibition and concentration of fungicides. Analysis of variance on conidial germination inhibition of

B. theobromae in different concentration of fungicides show that the items concentration, immersion period and concentration × immersion period were significant in every cases except Cupravit, Dithane M-45, Benamyl and Redomil MZ. LSD value also indicates significant different in conidial germination inhibition for every cases.

4.7.2 Effect of Different Plant-extraction on the Inhibition of Conidial Germination of *B. theobromae*.

The plant extracts were tested as fungicides and the results are presented in Table-21. Ten plant extracts were tested for the observation of inhibitory effect of *B. theobromae*. 95% and 70% conidial germination was inhibited after 30 minutes of immersion at 5:1.25(w/v) concentrations in *Tagetes patula* leaf and root extracts. In *Azadirachta indica* leaf and bark extracts and *Allium sativum* extracts 19, 35 and 20% conidial germination inhibited after 30 minutes of immersion. Among the plant extracts, *Cassia alata* leaf, *Leonurus sibiricus* leaf, *Vinca rosea* leaf, *Datura metel* leaf and seed extracts were less effective (3, 5, 8, 5 and 7% inhibition after 30 minutes immersion) on the inhibition of this fungus. Present study shows that there is an inactivation effect of plant extracts against *B. theobromae*.

The present study indicate that the inhibitory effect of the plant parts on conidial germination might be attributed to the presence of some antifungal ingredients in them, which can't totally inhibit the germination of *Botryodiplodia theobromae* as fungicides but they have some inhibitory effect on the fungus.

Range of correlation (r26) values is 0.8660 – 0.9999 indicate that there was highly significant relationship between conidial germination inhibition and immersion period. Analysis of variance on conidial germination inhibition of *B. theobromae* in different extracts show that the items extract, immersion period and extracts × immersion period were significant. LSD value also indicates significant different in conidial germination inhibition.

4.7.3 Effect of Different Concentrations of Spices extracts as Fungicides on the Inhibition of Conidial Germination of *B. theobromae.*

Three spices extracts were tested as fungicides in different concentrations and results are presented in Table-22. Three spices extracts were tested for the observation of inhibitory effect of *B. theobromae*. Hundred per cent conidial germination of *B. theobromae* was inhibited after 05-30 minutes of immersion at all concentrations in Ginger (*Zingiber officinale*) extracts and treated conidia were incubated upto 24 hours in humid chamber. On the other hand, Onion (*Allium cepa)* and Garlic (*Allium sativum)* extracts had antifungal effective, when the fungus was immersed for long duration and higher concentrations. Length of germ tube formation of the fungus was comparatively higher in Garlic extracted media than that of Onion extracted media is this experiment.

Ranges of correlation (r27) values –0.9002 to –0.8509 and –0.9605 to 9229 for *A. cepa* and *A. sativum* respectively indicate that there were highly negative significant relationship between concentration and percentage of conidial germination inhibition. Correlation (r28) values 0.9959 and 0.9943 for *A. cepa* and *A. sativum* respectively also indicate a highly significant relationship between incubation period and percentage of conidial germination inhibition. Analysis of variance on percentage of conidail germination of *B. theobromae* in different concentration of *A. cepa* and *A. sativum* show that the items concentration, incubation period and concentration × incubation period are significant in every case. LSD value also indicate significant different of conidial germination in every case.

4.7.4 Effect of Smoke on the Conidial Germination of *B. theobromae.*

Smoke of rice straw, wheat straw, tobacco leaf and dhup were effective for the inhibition of fungi. Wheat straw smoke inhibited 95% conidial germination of *B. theobromae* when this fungus was smoked using smoke for 15 minutes in a smoke chamber. Not only wheat straw, but also all tested smoke (rice straw, tobacco leaf and dhup) are effective for the inhibition (90, 78 and 61%) of conidial germination of *B. theobromae* in same period. It is evident from Table-23 that with the increase of exposure period, the rate of inhibition was increased.

Range of correlation (r29) values 0.9339 – 0.9843 indicate that there was highly significant relationship between conidial germination inhibition and smoke passing time. Analysis of variance on conidial germination inhibition of *B. theobromae* in different smoke shows that the items treatment, passing time and treatment × passing time were significant. LSD value also indicates significant different in conidial germination inhibition in different smoke.

4.7.5 Effect of Plant-Extracts with Cowdung on the Inhibition of Mycelial Growth of *B. theobromae* after 4 days of Incubation at 28±2°C.

Effect of plant-extracts incombination with cowdung on the inhibition of mycelial growth of *B. theobromae* is presented in Table-24.The present observation show that mycelial growth of *B. theobromae* was completely inhibited in ginger extracts with cowdung medium (20% ginger + 15% cowdung) on 4 days of incubation at 28±2°C. In case of neem extracts in combination with cowdung medium, 90mm growth was observed on 4 days of incubation on comparison with control (PDA-90 mm). There was no inhibitory effect of neem extracts against *B. theobromae*.

Correlation (r30) values 0.9755 and 0.9639 indicate that there was highly significant reationship between incubation period and mycelial growth. Analysis of variance on mycelial growth of *B. theobromae* in different media shows that the items incubation period, extracts and incubation period × extracts were significant. LSD value also indicates significant different in mycelial growth.

Table-20: Effect of different concentrations of fungicides on the inhibition of conidial germination of *B. theobromae* after immersed different period and observed after 24 hours of incubation at 28±2°C.

Name of Fungicides	Concentration (%)	Percentage of conidial germination[1] inhibition in different immersion period (minute)						Length of germ tube (μ) at 2o minute of immersion	Correlation (r24)
		5	10	15	20	25	30		
Cupravit	0.05	100	100	100	100	100	100	0	-
	0.10	100	100	100	100	100	100	0	-
	0.15	100	100	100	100	100	100	0	-
	0.20	100	100	100	100	100	100	0	-
	0.25	100	100	100	100	100	100	0	-
Thiovit	0.05	04	15	30	38	55	66	38.12-98.37	0.9938
	0.10	18	46	57	63	70	75	26.20-72.92	0.9339
	0.15	35	52	60	70	78	81	7.38-56.11	0.9827
	0.20	46	67	68	79	83	88	2.56-21.23	0.9659
	0.25	66	70	73	82	89	93	0.90-6.28	0.9837
Dithane-M-45	0.05	100	100	100	100	100	100	0	-
	0.10	100	100	100	100	100	100	0	-
	0.15	100	100	100	100	100	100	0	-
	0.20	100	100	100	100	100	100	0	-
	0.25	100	100	100	100	100	100	0	-
Rovral	0.05	32	55	70	83	76	79	20.52-110.70	0.9126
	0.10	64	66	69	75	80	82	16.30-76.35	0.9857
	0.15	77	79	80	81	83	85	5.60-53.38	0.9895
	0.20	82	84	87	88	89	90	4.45-21.25	0.9790
	0.25	85	87	90	91	92	93	0.80-5.24	0.9814
Benomyl	0.05	100	100	100	100	100	100	0	-
	0.10	100	100	100	100	100	100	0	-
	0.15	100	100	100	100	100	100	0	-
	0.20	100	100	100	100	100	100	0	-
	0.25	100	100	100	100	100	100	0	-
Bavistin	0.05	07	65	74	81	86	88	36.55-255.62	0.8375
	0.10	52	70	80	85	88	90	23.75-180.50	0.9481
	0.15	66	75	85	86	90	91	21.50-120.50	0.9556
	0.20	71	80	87	88	92	93	12.90-86.45	0.9645
	0.25	77	85	90	91	93	95	8.50-43.95	0.9632
Redomil MZ	0.05	100	100	100	100	100	100	0	-
	0.10	100	100	100	100	100	100	0	-
	0.15	100	100	100	100	100	100	0	-
	0.20	100	100	100	100	100	100	0	-
	0.25	100	100	100	100	100	100	0	-

Correlation (r25) - 0.9750, 0.9936 and 0.9646 for thiovit, rovral and bavistin respectively.

1 – Mean of three replications.

Correlation (r24) – Correlation between conidial germination inhibition and immersion period.

Correlation (r25) - Correlation between conidial germination inhibition and concentration of fungicides (average values).

Analysis of variance on percentage of conidial germination inhibition of *Botryodiplodia theobromae* in different concentration of Thiovit.

ITEMS	SS	DF	MS	F
Replication	29.5823	2	14.79115	405.1404*
Concentration	8832.903	4	2208.226	60484.92*
Immersion period	9360.983	5	1872.197	51280.84*
Concentration × Immersion period	829.5821	20	41.4791	1136.143*
Error	2.117504	58	0.036509	
Total	19055.17	89	214.103	

$LSD_{(0.05)}$ 0.312348

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on percentage of conidial germination inhibition of *Botryodiplodia theobromae* in different concentration of Rovral.

ITEMS	SS	DF	MS	F
Replication	35.63577	2	17.81788	783.0468*
Concentration	3817.073	4	954.2683	41937.45*
Immersion period	1499.457	5	299.8913	13179.39*
Concentration × Immersion period	836.8704	20	41.84352	1838.907*
Error	1.319764	58	0.022755	
Total	6190.355	89	69.55456	

$LSD_{(0.05)}$ 0.246589

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on percentage of conidial germination inhibition of *Botryodiplodia theobromae* in different concentration of Bavistin.

ITEMS	SS	DF	MS	F
Replication	43.30266	2	21.65133	578.0226*
Concentration	2684.104	4	671.0261	17914.29*
Immersion period	7153.008	5	1430.602	38192.57*
Concentration × Immersion period	2575.908	20	128.7954	3438.433*
Error	2.17254	58	0.037458	
Total	12458.5	89	139.9831	

$LSD_{(0.05)}$ 0.316381

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table-21: Effect of Different Plant Extracts as fungicides on the inhibition of conidial germination of *B. theobromae* immersing after 10, 20 and 30 minutes and 24 hours of incubation.

Name of plant extracts.	Percentage of conidial germination[1] inhibition in different immersion period (minute).			Correlation (r26)
	10	20	30	
1. *Casia alata* Leaf	0	0	3	0.8660
2. *Tagates patula* Leaf	21	48	95	0.9819
3. *Tagates patula* Root	15	32	70	0.9839
4. *Leonurus sibiricus* Leaf	0	0	5	0.8660
5. *Vinca rosea* Leaf	0	2	8	0.9999
6. *Datura metel* Leaf	0	0	5	0.8660
7. *Datura metel* Seed	0	2	7	0.9994
8. *Azadirachta indica* Leaf	0	6	19	0.9984
9. *Azadirachta indica* Bark	5	13	35	0.9856
10. *Allium sativum* Bulb	3	7	20	0.9771

1 - Mean of three replications.
Correlation (r26) – Correlation between conidial germination inhibition and immersion period.

Analysis of variance on percentage of conidial germination inhibition of *Botryodiplodia theobromae* in different immersion period in plant extracts.

ITEMS	SS	DF	MS	F
Replication	38.60508	2	19.30254	43.33945*
Extracts	20004.97	9	2222.774	4990.734*
Immersion period	7336.826	2	3668.413	8236.585*
Extracts × Immersion period	2217.814	18	123.2119	276.6443*
Error	25.83206	58	0.44538	
Total	29624.05	89	332.8545	

$LSD_{(0.05)}$ 1.090952
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table -22: Effect of different concentrations of spices extracts as fungicides on the inhibition of conidial germination of *B. theobromae* immersed after different period and observed 24 hours of incubation

Name of plants	Period of incubation (min.)	Percentage of conidial germination[1] inhibition in different concentrations (w/v)					Correlation (r27)	Length of germ tube (μ) at 5:3.5 (w/v) conc.
		5:1.5	5:2.5	5:3.5	5:4.5	5:5.5		
Onion (*Allium cepa*)	05	49	44	39	28	00	-0.8597	11.40-223.30
	10	57	48	44	35	00	-0.8509	12.80-291.55
	15	62	58	51	40	00	-0.8448	17.23-305.25
	20	65	61	57	46	00	-0.8297	19.20-350.30
	25	72	70	63	44	10	-0.9002	12.85-250.60
	30	75	71	68	55	17	-0.8802	22.40-235.15
Garlic (*Allium satium*)	05	54	50	33	12	00	-0.9605	98.25-496.55
	10	58	55	41	15	00	-0.9444	97.80-420.50
	15	65	60	52	19	00	-0.9287	93.60-315.50
	20	71	67	60	20	00	-0.9241	67.10-265.15
	25	77	74	62	29	04	-0.9229	18.80-212.50
	30	83	78	66	34	10	-0.9501	05.40-198.65
Ginger (*Zingiber officinale*)	05	100	100	100	100	100	-	-
	10	100	100	100	100	100	-	-
	15	100	100	100	100	100	-	-
	20	100	100	100	100	100	-	-
	25	100	100	100	100	100	-	-
	30	100	100	100	100	100	-	-

Correlation (r28) - 0.9859 and 0.9943 for Onion (*Allium cepa*) and Garlic (*Allium satium*) respectively.

1 = Mean of three replications

Correlation (r27) – Correlation between incubation period and percentage of conidial germination

Correlation (r28) - Correlation between concentration and percentage of conidial germination

Analysis of variance on percentage of conidial germination inhibition of *B. theobromae* in different concentration of Onion (*Allium cepa*) extracts.

ITEMS	SS	DF	MS	F
Replication	33.70506	2	16.85253	79.19161*
Incubation period	3451.599	5	690.3199	3243.878*
Concentration	25166.75	4	6291.688	29565.23*
Incubation period × Concentration	639.5437	20	31.97719	150.2638*
Error	12.34281	58	0.212807	
Total	29303.94	89	329.2578	

$LSD_{(0.05)}$ 0.754107

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Analysis of variance on percentage of conidial germination inhibition of *B. theobromae* in different concentration of Garlic (*Allium satium*) extracts.

ITEMS	SS	DF	MS	F
Replication	138.6306	2	69.31528	4.168518*
Incubation period	2882.724	5	576.5449	34.67256*
Concentration	35291.84	4	8822.959	530.5997*
Incubation period × Concentration	746.4132	20	37.32066	2.244409*
Error	964.4401	58	16.62828	
Total	40024.04	89	449.7084	

$LSD_{(0.05)}$ 6.665976

ns= Non significant at 5% level of probability.

*= Significant at 5% level of probability.

Table-23: Effect of smoke on conidial germination of *B. theobromae* previously grown on PDA, which was exposed to rice straw, wheat straw, tobacco leaf and dhup burnt for different duration of time.

Treatment	Percentage of conidial germination[1] inhibition after passing smoke for different times (minutes).			Correlation (r29)
	5	10	15	
Rice straw	53	61	90	0.9403
Wheat straw	57	69	95	0.9622
Tobacoo leaf	50	55	78	0.9339
Dhup	35	52	61	0.9843

1 – Mean of three replications.
Correlation (r29) – Correlation between conidial germination inhibition and smoke passing time.

Analysis of variance on percentage of conidial germination inhibition of *Botryodiplodia theobromae* in different time of smoke passing.

ITEMS	SS	DF	MS	F
Replication	8.809089	2	4.404544	54.34727*
Treatment	1304.614	3	434.8713	5365.837*
Passing time	2874.533	2	1437.267	17734.3*
Treatment × Passing time	302.5976	6	50.43293	622.2874*
Error	1.782978	22	0.081044	
Total	4492.337	35	128.3525	

$LSD_{(0.05)}$ 0.482086
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Table-24: Effect of Plant extracts with cowdung on the inhibition of mycelial growth of *B. theobromae* after four days of incubation at 28±2°C.

Period of incubation (days)	Radial growth of mycelium[1] in mm on different media.		
	Ginger extract+Cowdung	Nim extract+Cowdung	PDA (Control)
1	0	5	8
2	0	34	40
3	0	80	85
4	0	90	90
Correlation (r30)	-	0.9755	0.9639

1 – Mean of three replications.
Correlation (r30) – Correlation between incubation period and mycelial growth.

Analysis of variance on redial growth of mycelium of *Botryodiplodia theobromae* in different media (Plant extract + cowdung)

ITEMS	SS	DF	MS	F
Replication	8.166667	2	4.083333	9.135593*
Incubation period	18614	3	6204.667	13881.63*
Extracts	23401.5	2	11700.75	26177.95*
Incubation period × Extracts	9338.5	6	1556.417	3482.153*
Error	9.833333	22	0.44697	
Total	51372	35	1467.771	

$LSD_{(0.05)}$ 1.132145
ns= Non significant at 5% level of probability.
*= Significant at 5% level of probability.

Chapter 5

Discussion

DISCUSSION

Black band disease of jute (*Corchorus* sp.) is a serious fungal disease, caused by *Botryodiplodia theobromae*. This is a common disease of jute plant in the Northern part of Bangladesh. Factors influencing the development of disease and the pathogen have been studied in the present investigation. In addition to these, control measures of the pathogen have been developed.

Germination of conidia of *Botryodiplodia theobromae* was influenced by several factors. Temperature is an important factor for conidial germination of *B. theobromae*. In the present investigation, conidial germination occurred over the temperature range of 15°-40°C, but the optimum range was 25°-30°C at 1.5% glucose and sucrose solution. In case of 1:15(w/v) cowdung solution 100% germination took place at 35°C. Germination of conidia failed completely at 10° and 45°C in all the cases. Effect of temperature on conidial germination of chilli fruit rot pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed conidial germination over the temperatures range of 10°-35°C, but the optimum range was 20°-25°C. Meah (1993) observed that *B. theobromae* caused an infection of mango only when fruit started to ripen (soften at immature or mature stage). The highest rate of disease development occurred in temperature ranging from 25°-30°C and 10% fruit surface area/day was obtained at 32°C and the lowest at 1-3% fruit surface area/day at 13°C. No disease was observed at 10°C until 32 days after inoculation. Rewal and Grewal (1989) reported that minimum, optimum and maximum temperatures for the germination of conidia of all the three strains of *Botrytis cinerea* were 5°, 20° and 30°C. Singh and Chauhan (1988) found that maximum germination of zoospores of *Phytophthora drechsleri* was at 30°C and minimum was at 10° and 45°C. Vicente *et al.* (1983) observed

that ascospores germinated at 10°-35°C (1.8 and 22.5% respectively) and optimum at 25°C (99.2 %). During the study of *Altenaria porri*, Khare and Neema (1982) observed that cent percent spore germination occurred *in vitro* within four hours at 22°C, while maximum germination was recorded within six hours at 25°C on the host surface. Yadav and Agnihotri (1980) observed conidial germination of *Pyricularia penniseti* within 6 hours at 25°C on the moist surface. No difference in conidial germination of *P. penniseti* at 22°, 25° and 27°C in 12, 18 and 24 hours respectively, but the difference was marked at 6 hours. Spore germinated better at 25°C and the rate decreased rapidly above 27°C. Bhargava (1971) studied the effect of various environmental factors on the spore germination of *F. solani* and *Botryodiplodia ananassae*. He observed that spore germination of these two fungi was the best between 25°-30°C and it decreased with any increase or decrease in the temperature level. According to Walker (1969) the conidia of *Alternaria solani* germinated at the optimum temperature of 28°-30°C within 35 to 45 minutes. Singh and Singh (1951) reported that the minimum optimum and maximum temperature for germination of spore of *Ascochyta sorghi* was 25°C, 30°-32°C and above 38°C respectively. Hutchinson (1929) reported that 28°C as the optimum temperature for germination of spore of *B. theobromae*. The results of the present studies are in accordance with the earlier reports (Hutchinson, 1929; Bhargava, 1971 and Meah, 1993). Studies also revealed that germination of conidia of *B. theobromae* depends on variable temperatures, ranging between 15°-35°C, optimum being 25°-35°C; beyond which sharp decline takes place.

Relative humidity (RH) is an important factor for conidial germination of the fungus *B. theobromae*. The highest (99%) conidial germination was observed at 100% RH and the lowest (35%) was at 85%RH. Germination also initiated at 90% RH within four hours of incubation. There was no conidial germination recorded at 80% of RH. Effect of RH on conidial germination of chilli fruit rot

pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed that conidial germination was initiated in 2 - 6 hours at 90 and 75 % relative humidity. Jacome *et al.* (1991) reported that conidial germination of *M. fijiensis* var. *difformis* occurred over a wide range of RH, at 92-100 %, than that of ascospores at 98-100 % RH. Rewal and Grewal (1989) stated that three strains of *Botrytis cinerea* viz., B_1 needs 93-100%, strain B_4 81-100% and strain B_5 75-100 % RH for their conidial germination. Lim and Tang (1985) studied the growth and sporulation of *Colletotrichum gloeosporiodes* on Oatmeal agar and found that growth and sporulation are most abundant at 80-90% of RH. Vicente *et al.* (1983) reported that germination of ascospores of *Mycosphaerella musicola* takes place at RH > 88 %. The results of these studies are in accordance with the earlier workers, Vicente *et al.* (1983); Jacome *et al.* (1991) and it is concluded that conidia of *B. theobromae* shows variation in respect of RH requirements for germination.

Hydrozen ion-concentration (pH) affects greatly in the adaptability of organisms to their nutrient environment. In the present study, it was found that conidial germination of *B. theobromae* occurred at all the pH levels ranging from 2 to 10. The highest (83%) and the lowest (08%) conidial germination were found at 6 and 2 pH after 6 hours of incubation. The range of pH 5-7 was optimum for conidial germination. Moderate and poor type of germination recorded in rest of the pH level. In general fungal spores germinate within a wide range of pH. Chauhan and Singh (1991) found that zoospores of *Phytophthora drechslera* f. sp. *cajani* germianated in a wide range of pH (4.0-11.0). The maximum percentage of germination was observed at pH 7.5. Siddique and Mridha (1989) reported the effect of pH on growth and sporulation of *C. capsici,* a common pathogenic fungus of chilli (*Capsicum* sp.). They found that best growth and abundant sporulation are at around pH 5 in PDA medium. Du *et al.* (1988) stated that maximum growth of the fungus *M. rofidium* was at the range of pH 5.4-8.5 and suitable range at pH 4.0-9.5, when

cultured on PDA. Kumar and Singh (1987) studied that teliospores of *Neovossia horrida* in water for three days significantly increased the germination at pH 5.0. Mathur and Sarbhoy (1977) reported the maximum growth and excellent sporulation of *Sclerotium rolfsii* and *Alternaria alternata* at pH 5.5. Satya and Grewal (1972) found that *F. caeruleum* grew at different pH levels ranging from 2.0-8.0. No growth was obtained at pH 2.0. Optimum growth of the fungus was recorded at pH 6. Hawker (1950) stated that most fungi grow best at neutral pH that is at pH 7 or slightly on the acid side of the neutral. Smart (1937) reported that spores of *Fuligo septica* germinated from pH 2.0-10.0. The result in the present investigation resembles the work done earlier (Hawker, 1950; Satya and Grewal, 1972; Muthur and Sarbhoy, 1977; and Chauhan and Singh, 1991) and it concluded that conidial germination of *B. theobromae* affected by pH.

Conidial germination was observed in different percentage of glucose and sucrose solution of fungi *B. theobromae* after 12 hours incubation. The highest conidial germination was 1.5% glucose and 1% sucrose solution. Germination initiated on glucose and sucrose solution after 2 hours of incubation and germinated at all the concentrations within 12 hours. Conidial germination of *B. theobromae* also increased or decreased with the increase or decrease of glucose and sucrose. The rate of germination of conidia was higher on 1.5% glucose solution than that of 1.5% sucrose solution. Length of germ tube was measured higher on glucose solution than that of sucrose solution. Appaji and Thakur (1994) reported *in vitro* experimental result of pearl millet downey mildew by *Sclerospora graminicola* that sugar did not induce germination of spores. Glucose was found to show least inhibitory effect. They observed that as the concentration of sugars increased from 0.01 to 0.1 per cent and 1.0 per cent, the percentage of germination was reduced with all sugars except maltose, where in a marginal increased in sporangial germination was observed with increase in

concentrations from 0.01 to 0.1 per cent. Though inhibition was noted with increase in concentrations at the same time, it was also observed that the concentrations tested did not differ significantly from each other. The effects of dextrose (47.1%) and maltose (46.6%) were similar, so was sucrose (43.1%) and mannose (41.5%). Glucose alone was significantly different from rest of the sugars and it did not differ significantly with control. It induced higher percent germination (52.9). It is obvious from the results that sugars do not have any inhibitory effect on sporangial germination of *S. graminicola*. Jacome *et al.* (1991) studied that conidia of *Mycosphaerella fijiensis* var. *difformis* germinate maximum in free water. Chandra Mohanan *et al.* (1987) studied that the spore of *Colletotrichum gloeosporioides* germinated maximum in tap water. Kore and Kharwade (1987) observed that 50 % germination occurred in Tinda fruit juice followed by 2% sucrose solution (32 %) respectively. They also found that, of different media like, Tinda fruit juice, 2% sucrose solution, sterile water, tap and distilled water are sufficient for conidial germination. Khare and Neema (1982) observed that increasing concentration of sucrose inhibited germination of *Alternaria porri* spore. Lin (1945) while working on *Glomerella cingulata* found that for successful germination of spores required carbohydrate, nitrogen, phosphorus and magnesium. From this result, it is amply clear that glucose is essential to help the maximum germination of conidia of *B. theobromae*.

Effects of different concentrations of cowdung solution on conidial germination of *B. theobromae* after 2-12 hours was observed in the experiment. 1:1.5 (w/v) concentration gave the highest germination (100%) of conidia. Germination of conidia was initiated on all concentrations cowdung solution after 4 hours of incubation and germinated in all the concentrations within 12 hours (Table-6). In the present observation, it may be concluded that *B. theobromae* can freely grow on cowdung solution, 1:15(w/v) cowdung concentration showed 100%

conidial germination after 12 hours of incubation. The length of germ tube (51.4μ–α) was measured on cowdung solution after 12 hours of incubation.

Conidial germination of *B. theobromae* is affected through different water. Conidial germination of *B. theobromae* was the highest (93%) in river water and the lowest (44%) in tap water on the other hand, in rain, distilled and pond water conidial germination was recorded 90,88 and 65% which was better than sterilized water. Jacome *et al.* (1991) observed that conidia of *Mycosphaerella fijiensis* var. *difformis* germinate maximum in free water. Pure water is sufficient for the germination of spores of many fungi, but some require an additional supply of nutrients or other substances for successful germination. Chandra Mohanan *et al.* (1987) studied that the spores of *Colletotrichum gloeosporioides* germinate maximum in tap water.

Effect of different concentrations of KNO_3 and $NaNO_3$ salt solution on conidial germination of *B. theobromae* after 2-24 hours is shown in Table-8. In both the cases, conidial germination was initiated in 4 hours of incubation. After 24 hours of incubation, the highest conidial germination was recorded in 1% KNO_3 and $NaNO_3$ (48 and 88%) salt solution. 1% concentration of KNO_3 and $NaNO_3$ resulted better germination as compared to other concentrations of KNO_3 and $NaNO_3$ salt solution used in this experiment. In every cases, germination of conidia was the lowest (13 and 40%) in 3.5% concentration in both the salt solution. Rest of the concentration of KNO_3 had poor effect and $NaNO_3$ had moderate effect on germination of conidia. In case of 2.5, 3.0 and 3.5% of KNO_3 salt solution, conidial germination started on 6^{th} and 8^{th} hours of incubation. Alam *et al.* (2002) reported that conidial germination of *Bipolaris sorokiniana* was the highest (100%) in 1.5% and the lowest (5%) in 3.5% KNO_3 solutions within 48 and 2 hours of incubation period respectively. In $NaNO_3$ the highest (83%) conidial germination was recorded in 1% and the lowest (3%) in 3.5% solution

within 48 and 2 hours of incubation period. With the increase or decrease of KNO_3 in solution the rate of germination was increased or decreased. But in $NaNO_3$ solution, conidial germination was gradually decreased with the increase of $NaNO_3$ in solution.

Effect of different solid media on mycelial growth, dry weight and sporulation of *B. theobromae* has been studied. The mycelial growth was the highest (90 mm) in PDA medium and the lowest (64 mm) in Richard's medium on 4 days of incubation. The dry weight of mycelia was the highest (220 mg) in PDA medium and the lowest (110 mg) in Richard's medium after 15 days of incubation. Sporulation of *B. theobromae* was also inadequate number (96/0.01ml) in Richard's medium and inadequate (24/0.01ml) in PCM medium. Quroshi and Meah (1991) observed that *Botryodiplodia theobromae* grows faster on Richard's agar medium and poorest on Czapek's agar meduim. Siddique and Mirdha (1989) studied that *C. capsici* grew and sporulated well in PDA. Chandra Mohanan *et al.* (1987) reported that incase of *C. gloeosporioides*, Oat-meal-agar was the best medium for germination and degree of infection. Conidial germination was maximum in tap water and also the degree of infection. Ahmed (1985) observed that growth and sporulation of *Colletotrichum gloeosporioides* were well both on PDA and its host fruits (mango) medium. Jhamaria (1972) reported that different media showed marked variability in growth, sporulation and colony characters of *Fusarium oxysporum* potato-dextrose-agar, Richards agar and Czapek's agar media provided maximum growth and sporulation of the fungus. Konger (1971) observed that among all the media tried, potato-dextrose agar was found to be most suitable for growth of mycelium in *Fusarium oxysporum* and *F. radicicola.* Booth (1971) preferred to maintain the culture of *Fusarium* on potato-dextrose-agar because *Fusarium* isolates have a remarkable faculty for adapting both their form and colour in response to the pressure of cultural environment. Toussoun *et al.* (1960)

demonstrated in case of *F. solani* f. sp. *phaseoli* on enhanced nitrogen while inoculum was growth on organic nitrogen and while inoculum growth on the media containing glucose delayed pathogenesis. It also indicates that there was direct relationship between the colony growth and sporulation of the fungus in PDA. Gordon (1952) in Winnipeg found potato sucrose agar eminently suitable for *Fusarium* culture and this is the standard medium used at the CMI Kew. The present study indicates that nutrients are the sources, which can stimulate the growth and sporulation of the fungi.

Effects of different concentrations of solid cowdung media on mycelial growth of *B. theobromae* on 4 days was recorded and shown in Table-10. Results showed that growth of mycelium of *B. theobromae* was the highest (90) at 20% concentration of cowdung and PDA (control) media. 15 and 10% media had intermediary effect on mycelial growth (74 and 66 mm) of this fungus. In 5% cowdung medium, mycelial growth was lower (34 mm). It was observed form Table-12 that the highest dry weight (330 mg) was measured on 15% cowdung medium and the lowest dry weight (135 mg) was on 5% cowdung medium after 15 days of incubation. The highest (94/0.01 ml) sporulation of the fungus was recorded at 20% cowdung medium and the lowest (53/0.01 ml) was in 10% cowdung medium. There was no conidium of the fungus found in 5% cowdung medium. Basak *et al.* (2002) studies on comparative efficacy and *in vitro* activity of cow urine and cow dung for controlling root rot disease of cucumber caused by *Fusarium solani* f. sp. *cucarbitae* Snyder & Hansen following slide germination and mycelial growth inhibition tests. They observed that both germination of conidia and the percentage inhibition of mycelial growth decreased or suppressed and varied greatly with respect to different hour and days of incubation and kind of bio-matters. In this test cow dung potato dextrose agar (CDPDA) had less efficacy in suppression of the percentage inhibition of mycelial growth.

Effects of different concentrations of fertilizers (urea, gypsum potash, boron, zinc and sulpher) in combination with PA media on the mycelial growth and dry weight of *B. theobromae* have been studied and results are presented Table-13. Mycelial growth of *B. theobromae* was measured the highest in boron fertilizer medium (90 mm) than that of other tested media. Results showed that growth of mycelium of *B. theobromae* was the highest (90 mm) in 3% concentration of boron fertilizer and the lowest (45mm) in 3% concentration of potash fertilizer media after 4 days of incubation. On the other hand, except sulpher containing media, rest of the tested media had intermediary effect on mycelial growth. It is evident from the study that increase of fertilizer in the media, the growth of mycelium of the fungus was increased. In case of sulpher containing media, the highest mycelial growth (65 mm) was observed in 2% medium. The rate of mycelial growth was decreased with the increase or decrease of sulpher concentrations in the medium. Results show that the dry weight of mycelia was the highest (240 mg) in 3% concentration of boron containing medium and the lowest (50 mg) in 3% concentration of urea and gypsum media.

Effect of different concentrations of mustard and linseed oil-cake on the mycelial growth of *B. theobromae* has been studied and results are shown in Table-14. Result shows that the mycelial growth of *B. theobromae* was increased with the increase of concentrations of both mustard and linseed oil cake media. The highest mycelial growth (90 mm) was measured in 10, 15 and 20% concentrations of mustard oil-cake medium and the lowest (80 mm) in 5% concentration of mustard oil-cake medium after 4 days of incubation. On the other hand, the highest mycelial growth of *B. theobromae* was measured (90 mm) in 15 and 20% concentrations and the lowest (67 mm) in 5% concentration of linseed oil cake. Mycelial growth of the fungus was 80, 90, 90 and 90 mm recorded in 5, 10, 15 and 20% concentrations of mustard oil-cake medium. Whereas 67, 78, 90 and

90 mm of mycelial growth were found in 5, 10, 15 and 20% concentrations of linseed medium. It is evident from the experiment that mustard oil-cake is more favorable for the growth of *B. theobromae* than that of linseed oil-cake. The highest dry weight of mycelia (340 mg) was measured in 20% concentration and the lowest (170 mg) at 5% concentration of mustard oil-cake medium.

Effect of different concentrations of KNO_3 and $NaNO_3$ in combination with potato-agar (PA) media was tested for mycelial growth and dry weight of *B. theobromae* on 7 days of incubation and the results are presented in Table-15. It is observed from Table-15 that in PA containing KNO_3 media, mycelial growth and dry weight was faster only (82 mm and 120 mg) and slower on 3.5% KNO_3 added PA medium (39 mm and 10 mg). Mycelial growth rate and dry weight were gradually decreased with the increase of KNO_3 in the media. On the other hand, $NaNO_3$ containing PA media the highest mycelial growth and dry weight were observed only on PA medium (80 mm and 110mg) and the lowest on 3.0% $NaNO_3$ added PA medium (08 mm and 20 mg). Growth and dry weight of this fungus gradually decreased with the increase of $NaNO_3$ concentrations. No mycelial growth was found on 3.5% of $NaNO_3$ added PA medium. Hebbar *et al.* (1996) observed that the mycelial growth and conidia and chlamydospore formation were different for the 3 strains and varied with the substrate used. The quantity of conidia and chlamydospores produced depended on the concentration of the substrates. Irrespective of carbon: nitrogen ratios, chlamydospores were formed rapidly in liquid media and in greater amount on substrates with a low utilizable carbon content (aqueous extracts of soyabean hull fibre and corn cob) than on those with higher utilizable carbon content (potato dextrose broth, aqueous extracts of cotton seeds and molasses yeast extract broth). In liquid cultures, increases in the concentration of substrates high in utilizable carbon resulted in reduced chlamydospore formation, however, this was far less in those with a lower

carbon content. Rahman *et al.*(1993) reported that effect of nitrogen and carbon sources on growth of *Fusarium oxysporum* and *Sclerotium rolfsii* and observed the effects of 5 inorganic and 10 organic N sources and of 12 C sources on *F. oxysporum* and *S. rolfsii.* El-Abyad and Afifi (1989) observed that growth and sporulation of *F. oxysporum* f.sp. betae in its saprophytic phase, were likely to reach opt. within the temperature range 25-30°C. Sporulation was maximum at 100% RH. Growth was highly enhanced in acidic sandy-clay soils (50:50, w/w) adjusted to 40-60% of its moisture holding capacity. Acidic reactions were favourable for the population of macroconidia. A sucrose concentration of 3% stimulated growth and sporulation. Although growth was favoured by the presence of 0.8% concentration of organic nitrogen source (asparagine), sporulation was favoured by the presence of 1% $NaNO_3$. A balanced high C: N ratio (1.5:1.5) stimulated both growth and sporulation of the fungus. Ghosh and Sen (1973) reported that nitrogen requirements of four isolates of *Macrophomina phaseoli* (Maubl.) Ashby. They found that all 4 isolates of *M. phaseoli* (*M. phaseolina*) from jute utilized ammonium, nitrate, nitrite and organic N. Some amino acids were better N sources than inorganic ones. A wide range of N conc. was utilized when there was sufficient C present. N was not the important factor in increasing growth when the C/N ration was changed.

The effect of different plant extracts on mycelial growth of *B. theobromae* was tested and the results are presented in Table 16. The highest mycelial growth (90 mm) was found in treatment with *Euphorbia hirta* (after four days of incubation) and the lowest (20 mm) in *Datura metel* and *Polygonum orientale.* The mycelial growth in *Lantana camara*, *Cucurbita* sp, *Tagetes patula* media were intermediary and rest of the other tested media had moderate effect. The highest dry weight (200 mg) of mycelium was measured in *Euphorbia hirta* and the lowest (10 mg) in *Datura metel* and *Clerodendrum viscosum* medium, in rest

of the tested media (*Ocimum sanctum*, *Vinca rosea*, *Adhatoda vasica*, *Rauvolfia serpentina*, *Zizyphus jujuba*) the dry weight of this fungus was intermediary (170,160,150,120 and 110mg). And other tested media had moderate effect of dry weight. Sindhan *et al.* (1999) reported the effect of some plant extracts on the vegetative growth of root rot causing fungi *Rhizoctonia solani* and *Rhizoctonia bataticola*. They found that extracts of all the plants were toxic to the mycelial gowth of *R. solani* and *R. balaticola* and bulb and rhizomes extracts of *A. cepa*, *A. sativum*, *Z. officinales* and leaf extracts of *A. indica* were more toxic to both the fungi as compared to other extracts. Basak and Paul (1999) has observed that plant extracts of *Azadirachta indica*, *Polygonium hydropiper*, *Lantana camara*, *Cassia tora* and *Moringa oleifera* had suppressive effect on mycelial growth of six major fruit rot fungal pathogens of chilli. Srivastava and Lal (1997) observed an *in vitro* test indicated fungicidal properties in aqueous leaf extracts of *Calotropis procera*, *Azadirachta indica*, *Lantana camara* and *Ocimum basilicum* aginst *Curvularia tuberculata* and *Alternaria alternata*. The fungi-toxicity or antifungal activity of leaf extracts of *Azadirachta indica, Chromolaena odorata, Lantana camara, Piper colubrinum* and *Strychnos muxvomica* have been reported by Anandarj and Leela (1996) on the different growth phases of *Phytophthora capsici*. They found that mycelial growth, sprongial production, zoospore production and release, and zoospore germination were completing by *C. odorata* extracts at 2% concentration. *A. indica* extracts also acted similarly but mycelial growth was inhibited only by 75.5%. *P. colubrinum* extracts inhibited mycelial growth and sporangial production whereas, sporangial germination and zoospore germination were inhibited by 23.71 and 20.13 % even at 2 % concentration. The extracts of *S. nuxvomica* had inhibitory effect on sporangial production at 0.25 % concentration whereas, on the other phases of the fungus, it was not very effective. The extracts from *L. camara* were effective. Tewari and Dath (1984) observed the effect of

leaf extract media of some plants on the growth of three fungal pathogens of rice. Only rice leaf extract medium favoured the growth as well as sporulation of *Drechslera oryzae.*

The mycelial growth and conidial counts at different temperatures after three and fifteen days of incubation are presented in Table-17. It was observed that the temperatures range of 25°-30°C was optimum for mycelial growth of *B. theobromae* on PDA (78-90 mm) and cowdung (80-90 mm) media. But rest of the temperatures had intermediary effect. The relative abundance of the mycelia increased with the increase of temperature. In both the media, growth and sporulation of the fungus was found to be inhibited at 45°C. There was no mycelial growth and sporulation at temperature 10° and 45°C. Sporulation occurred at 15°-40°C. In both media, the highest sporulation occurred at 30°C (38 conidia/0.01 ml and 42 conidia/0.01 ml) and the lowest was at 15°C (5 conidia/ 0.01 ml and 7 conidia/0.01 ml). Alam *et al.* (2001) reported the effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botryodiplodia theobromae* Pat. They found that the growth and sporulated at 10°-40°C and optimum range was 25°-30°C of this fungus. Effect of various culture conditions on *A. alternata* and *F. oxysporum*: 1. culture media, temperature, age and carbon source have been reported by Osman *et al.* (1992). They found that optimum conditions were achieved by incubating cultures for 8 days at 30°C using sucrose as the carbon source. Ahmed (1985) observed that growth and sporulation of *Colletotrichum gloeosporioides* were well both on PDA and its host fruits (mango) medium. They found temperature and light enhances the growth and the number of conidia formation.

Light also has some effect on *Botroydiplodia theobromae.* PDA medium was exposed to light for different time for the studies of mycelial growth and sporulation of *Botroydiplodia theobromae.* The highest growth and sporulation

was observed in continuous light condition and the lowest growth was observed in 8 hours light and 16 hours darkness, sporulation was lowest in continuous darkness. Alam *et al.* (2001) reported the effect of temperature, light and media on growth, sporulation, formation of pigments and pycnidia of *Botroydiplodia theobromae* Pat. They found that light was not necessary for growth, but it enhance the sporulation of this fungus. Effect of light on mycelial growth of chilli fruit rot pathogen *Alternaria tenuis* has been reported by Alam *et al.* (1998). They observed that mycelial growth of *A. tenuis* was the highest in continuous light and the lowest in complete darkness. Chauhan and Singh (1991) found that light had also marked inhibitory effect on zoospore germination of *Phytophthora drechsleri* f. sp. *cajani*. Maximum germination (84.7%) was obtained in complete darkness. The reduction in germination ensured following exposure of zoospore to light of low intensity but at 500 lux the germination was completely inhibited. The observation recorded in the present work resembles the work done earlier and showed light had marked variations of germination of spores of *F. oxysporum* f. sp. *vasinfectum*. Rewal and Grewal (1989) reported conidia germination of *Botrytis cinerea*, strain B1 in continuous light, strain B4 in alternating 12 hours of light/darkness and strain B5 in complete darkness. Tiwari and Yadav (1977) reported that vegetative growth was more in violet and green light while sporulation was more in red and yellow light in two strains of *Choanephora cucurbitarum*. Vegetative growth and sporulation were minimum in continuous dark and continuous artificial light. Booth (1971) observed that diffused light favored spore germination in *Fusarium* species.

Viability of conidia of *B. theobromae* was tested under laboratory condition and results are presented in Table-19. The viability of *B. theobromae* was remained 100% up to 2 months of storage at room temperature (28±2) °C. It is evident from the experiment that after 2 months of storage, the viability of conidia

began to lose. 97-54% viability of conidia remained from 3-8 month of storage. After 10 months of storage, conidia of *B. theobromae* showed 38% viability and the experiment was not further continued. Verma (1972) reported that the spores of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were viable upto 11 months. Spore germination was at best 25°C in case of *Colletotrichum gloeosporioides* and at 30°C in case of *Botryodiplodia theobromae*.

Of the seven fungicides tested (Table-20), all were not effective against *B. theobromae,* when the fungus was immersed for 5 to 30 minutes at 0.05-0.25% solution. Thiovit, Rovral, and Bavistin have no effect against *B. theobromae*. Cupravit, Diathane-M-45, Benomyl and Redomil-MZ were effective against *B. theobromae*. Alam *et al.* (2002) reported the *in vitro* inhibition of conidial germination of *Colletotrichum gloeosporioides* Penz. and observed that dithaneM-45, rovral, thiovit and suncozeb were most effective against *C. gloeosporioides* after 5-30 minutes immersion at 0.05%-0.25% concentration. Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling purple blotch of onion seed-crop and observed that combined application of rovral 50wp @ 0.2% + redomil MZ-72 @ 0.2 % gave the best control of purple blotch and maximum seed yield of onion followed by individual application of rovral 50wp @ 0.2 % and score 250EC @ 0.05 % when sprayed at an interval of 15 days. Alam *et al.* (2000) reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found bavistin, dithane M-45 and tilt were the most effective fungicides. They stated that at 500 to 2500 ppm and 1/10 to 1/1000 ml concentrations were most effective on the fungus after 5 to 30 minutes immersion. Alam *et al.* (1999) reported the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found that redomil, dithane M-45, cupravit, bavistin and rovral proved to be the most effective against *A. tenuis* when immersed for 5 to 30 minutes at 500 to 2500 ppm concentrations. Sharma and Gupta (1994) reported that 6

fungicides namely carbendazim bitertanol, traidimefon, trideorph, triforine and thiophenate methyl significantly inhibited spore germination of *Podosphaera leucotricha* and reduced germ tube length. The maximum inhibition (94%) was obtained in triforine and minimum (72%) in thiophanate methyl at 500ppm. Antisporulant activity of the fungicides was observed up to 14 days in all except carbendazim and bitertanol, where there was sporulation up to 21 days. In the field bitertanol followed by carbendazim and triforine provided the best control of mildew. Ahmed *et al.* (1991) evaluated eight fungicides and observed Dithane M-45 (Suncozeb) to give the best control of anthracnose (*Colletotrichum gloeosporioides* Penz.) followed by Bordeauxe mixture. Singh *et al.* (1990) reported control of leaf spot caused by *Alternaria brassicae* under field conditions by 7 fungicides, namely emisa-6, bavistin, captafol, cumin-L, difolatan, dithane M-45 and dithane Z-78 and suggested economically viable control measures acceptable to farmers. Siddaramaiah and Hegde (1990) reported that out of ten fungicides tested in *in vitro,* bavistin completely inhibited the growth of *Cercospora moricola* at 1000 ppm. Next best was benomyl followed by dithane M-45, aureofungin sol, dithane Z-78, and decolin. Under field condition, out of ten fungicides tested, bavistin (0.05%) was found to be highly effective in checking *Cercospora* leaf spot of mulberry followed by dithane M-45 (0.02%) and decolin (0.02%). Tewari *et al.* (1988) evaluated minimum inhibitory concentration of 5 fungicides as bavistin, benlate, dithane M-45, tecto-40 and calixin using the poisoned food technique *in vitro* and all except dithaneM-45 was also tested *in vivo*. Bavistin and tecto-40 were most effective *in vitro* and gave 95 and 85% control, respectively. Dithane M-45 was not effective. Benlate and calixin gave 70 and 80% control, respectively. Mehta *et al.* (1986) reported that potassium metabisulphate at 600 ppm, ascorbic acid at 1100 ppm, naphthalene acetic acid at 200 ppm and bavistin at 2000 ppm spray done 15 years old Dashehari and Chumsa

mangoes effectively controlled malformation. Bavistin proved most effective with 95 to 91.3% disease reductions respectively. Khanna and Chandra (1975) studied *in vitro* and found that benomyl and aretan were highly toxic to the growth of *Fusarium moniliforme* and *Fusarium roseum*. Aureofungin is found effective only at higher concentration. The fruit treated with benomyl retained their edible quality.

Plant extracts have been used in the present study for the test of inhibitory effect of conidial germination. Ten plant extracts viz., *Casia alata* leaf, *Tagates patula* leaf, *Tagates patula* root, *Leonurus sibiricus* leaf, *Vinca rosea* leaf, *Datura metel* leaf, *Datura metel* seed, *Azadirachta indica* leaf, *Azadirachta indica* bark, and *Allium sativum* bulb were used for the inhibition of *B. theobromae*. *Tagates patula* leaf inhibited 95 per cent conidial germination of this fungus. *Tagates patula* root inhibited better germination and rest of the plant extracts has poor effect on the inhibition of the fungus. The present study showed that conidial germination was increased or decreased with the increase or decrease of incubation period. Alam *et al.* (2002) reported the antifungal activities and (*in vitro*) inhibition of spore/ conidial germination of four fungi viz., *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae* was tested using the extracts of different parts of *vinca rosea* and *Azadirachta indica*. They observed that *Vinca rosea* root and *A. indica* (Leaf, root and seed) extracts showed good (100%) inhibition results on *B. sorokiniana* and *R. artocarpi* when it was immersed from 5-30 minutes at 5:1.25 ml (w/v) concentration. Alam *et al.* (2002) evaluated ten plant extracts conciderded as fungicides and observed *Tagetes erecta* leaf and *Azadirachta indica* bark extracts were the most effective for conidial germination inhibition of *Colletotrichum gloeosporioides* Penz. after 5-30 minutes of immersion in 5:1.25 ml (w/v) concentration. Alam *et al.* (2000) reported the growth inhibition of

Bipolaris sorokiniana by using different parts of *Allium cepa, Allium sativum, Zingiber officinale, Curcuma longa, Nigella sativa, Datura metal, Tagestes tulda, Eucalyptus citriodera, Lawsonia alba, Euphorbia pulcherima, Calotropis procera, Moringa oleifera, Ocimum sanctum, Psidium guajava, Vinca rosea, Striga densiflora* and *Eclipta alba.* They found, 100 % growth of *B. sorokiniana* was inhibited in *L. alba* at 5 and 10 % concentration after 7 days of incubation. They also observed germ tube formation inhibited in *D. metal, P. guajava, A. sativum* and *S. densiflora* than other plant extracts tested. Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *Vinca rosea* and leaf, root and seed extracts of *Azadirachta indica* against chilli rot pathogen *Alternaria tenuis.* Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *Botryodiplodia theobromae, Fusarium oxysporum, Helminthosporium spiciferum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum.* They used some medicinal plants viz., *Calotropis procera, Vitex negundo, Thuja orientalis, Argemone mexicana, Achyranthes aspera, Datura fastusa* and *Ricinus communis* and observed good control against these pathogens. Of the 11 leaf extracts, those of *Azadirachta indica* and *Occimum sanctum* were most effective in controlling the fungi. Chauhan and Joshi (1990) reported that the efficacy and persistence of 14 plant extracts and carbendazim as mango fruit diptreatments were comparedin controlling mango fruit anthracnose (caused by *Colletotrichum gloeosporioides*), Carbendazim (0.055) was the most effective control treatment. Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for 72 weeks when fruit was inoculated and was significantly batter than the other plant extracts tested. Caster oil (5%), eucalyptus oil (1%), garlic bulb, zingiber officinale, mango, turmeric and lantana leave also significantly controlled the disease. Persistence was max with carbendazil (0.05%) even in the pulp followed by castor oil (10%), garlic bulb and ardusi leaves. Carbendazil leaf resides in the pulp but plant extracts

only persisted in the pericarp. The present study indicates the presence of some antifungal compound in *Tagates erecta* leaf and *Tagates erecta* root.

Three spices extracts were tested as fungicides in different concentrations and results are presented in Table-22. Three spices extracts were tested for the observation of inhibitory effect of *B. theobromae*. Hundred per cent conidial germination of *B. theobromae* was inhibited putting after 05-30 minutes of immersion at all concentrations in Ginger (*Zingiber officinale*) extracted at 24 hours of incubation. On the other hand, Onion (*Allium cepa)* and Garlic (*Allium sativum)* extracts had antifungal effective, when the fungus was immersed for long duration and higher concentrations. Length of germ tube formation of the fungus was comparatively higher in Garlic extracted media than that of Onion extracted media is this experiment.

Botryodiplodia theobromae grown on previously exposed to rice straw, wheat straw, tobacco leaf and dhup smoke for different length of time was recorded in Table -23. Wheat straw inhibited 95% conidial germination of *B. theobromae* when this fungus was smoked using smoke for 15 minutes in a smoke chamber. Not only wheat straw, but also all tested smoke (rice straw, tobacco leaf and dhup) are effective for inhibition (90, 78 and 61%) of conidial germination of *B. theobromae* in same period. Alam *et al.* (2002) reported the antifungal activities (*in vitro*) of inhibition of spore/conidial germination of four fungi viz., *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae* by using smoke of rice straw, wheat straw, tobacco leaf straw and 'dhup' (incense) and observed that smoke had a great antifungal effect against these fungi. Alam *et al.* (1999) reported similar result against chilli fruit rot pathogen *Alternaria tenuis*. They observed that growth of *A. tenuis* was totally inhibited when inoculated on the medium exposed to rice

straw smoke and dhup smoke for 5-15minutes. Tobacco leaf smoke was ineffective against *A. tenuis* as fungitoxicides.

Effect of plant-extracts in combination with cowdung extracts on the inhibition of mycelial growth of *B. theobromae* presented in Table-24. The present observations show that mycelial growth of *B. theobromae* was completely inhibited on ginger extracts in combination with cowdung medium (20% ginger + 15% cowdung) after four days of incubation at 28±2°C. In case of neem extracts in combination with cowdung medium no inhibitory effect against *B. theobromae* was observed.

Chapter 6

Summary

SUMMARY

Botryodiplodia theobromae Pat. causing " Black band" disease of jute was found to be prevalent in the Northern region of Bangladesh. Factors influencing the development of pathogen and disease have been studied in the present investigation.

The conidia of *Botryodiplodia theobromae* was acrogenous, hyaline when young, later becoming medially one euseptate, dark brown, thick-walled, ellipsoid, base truncate, with longitudinal striation from apex to base, paraphysis hyaline, cylindrical septet.

Conidial germination of *B. theobromae* was influenced by several factors. In the present investigation conidial germination of *B. theobromae* occurred over the temperature range of 15°-40°C, but the optimum range was 25°-30°C in 1.5% glucose and sucrose solution. On the other hand, in cowdung extracts (1:15 w/v) it was 35°C. No germination occurred at 10° and 45°C.

Relative humidity is another important factor for conidial germination of *B. theobromae.* The present study shows that conidia of this fungus germinate better at higher relative humidities (RH), ranging from 80 to 100%.

Conidial germination of *B. theobromae* occurred within a pH range of 2 to 10, and there was a clear optimum pH at 6 to 7.

The rate of germination of conidia of *B. theobromae* was higher on 1.5% glucose solution than that of sucrose solution. In cowdung solution, 1:15(w/v) concentration gave the highest germination of *B. theobromae* conidia. On the other hand, 1% KNO_3 and $NaNO_3$ salt solution gave better germination of *B. theobromae* conidia. Length of germ-tube was measured higher on glucose solution than that of other tested solution after 12 hours incubation at room temperature.

After 8 hours of incubation, the highest germination was recorded in river water (93%) followed by rain water (90%), distilled water (88%), pond water (65%), sterilized water (54%) respectively and the lowest being (44%) in tap water.

Mycelial growth of *B. theobromae* on different media in petriplate culture was measured after four days of incubation and the highest growth was observed in PDA and cowdung media (90 mm) and lowest in Richard's (64 mm) medium. Dry weight of mycelia was the highest in PDA medium (220 mg) and the lowest in Richard's medium (110 mg). Sporulation was the highest in Richard's medium (96/0.01 ml) and the lowest in PCM medium (24/0.01 ml). Dry weight and sporulation was measured after 15 days of incubation.

Mycelial growth of *B. theobromae* in different concentrations of cowdung extracts media in petriplate culture was measured after four days of incubation and the highest growth was observed in 20% cowdung medium (90 mm) and the lowest 5% cowdung medium. Dry weight of mycelia was the highest in 15% cowdung medium (330 mg) and the lowest in 5% cowdung medium (135 mg). Sporulation was the highest in 20% cowdung medium (94/0.01 ml) and there was no conidia found in 5% cowdung medium. Dry weight and sporulation was measured after 15 days of incubation.

Mycelial growth of *B. theobromae* was measured after 4 days of incubation and the highest growth (90 mm) was observed in 3% concentration of boron fertilizer and the lowest (45mm) in 3% concentration of potash fertilizer media. Dry weight of mycelia was the highest (240 mg) in 3% concentration of boron containing medium and the lowest (50 mg) in 3% concentration of urea and gypsum media.

Mycelial growth of *B. theobromae* on mustard and linseed oil-cake media was observed after 4 days of incubation and the highest mycelial growth (90 mm) was measured in 10, 15 and 20% concentrations of mustard oil-cake and the lowest (67 mm) in 5% concentration of linseed oil cake media. The highest dry weight of mycelia (340 mg) was measured in 20% concentration and the lowest (170 mg) at 5% concentration of mustard oil-cake medium.

Mycelial growth of *B. theobromae* on $NaNO_3$ and KNO_3 with PA in petriplate culture was measured after seven days and the highest growth observed in PA medium and the lowest in 3% $NaNO_3$ media. Dry weight of mycelia was the highest (120 mg) in PA medium and the lowest (10 mg) in 3.5% KNO_3 medium.

Mycelial growth of *B. theobromae* in different plant extracts media after seven days was measured and the highest (90 mm) growth in *Euphorbia hirta*, *Moringa olifera*, *Acalypha indica*, *Rauvolfia serpentina*, *Zizyphus jujuba* and *Adhatoda vasica* and the lowest (20 mm) in *Datura metel* and *Polygonum orientale* plant extracts media were observed. Dry weight of mycelia was the highest in *Euphorbia hirta* (200 mg) and the lowest in *Datura metel* and *Clerodendrum viscosum* (10 mg).

Mycelial growth and conidial counts of *B. theobromae* were carried out at different temperatures (10°, 15°, 20°, 25°, 30°, 35°, 40° and 45°C) after three and fifteen days of incubation. The optimum temperature for mycelial growth on PDA and cowdung media at 30°C.

The growth of *B. theobromae* is affected much by the different light condition on PDA medium. The highest growth was observed in continuous light condition. However, for good conidia production in continuous light and alternate light and darkness was needed.

Viability of conidia of *B. theobromae* was 100% per cent up to 2 months of storage under laboratory at room temperature (28±2 °C). But after 2 months of storage, the conidia began to lose their viability.

Out of seven fungicides tested, Cupravit, Benomyl, Redomil-MZ and Diathane M-45 were most effective fungicides in the inhibition of *B. theobromae* after 5 to 30 minutes immersion. Thiovit, Rovral and Bavistin were not effective against *B. theobromae.*

Ten plant-extracts tested for their fungicidal efficacy, two were most effective against *B. theobromae*, three were less effective and five were not effective against *B. theobromae*, when the fungus was soaked for 10 to 30 minutes at 5: 1.25 (w/v) concentrations.

Out of three spices tested for fungicidal efficacy, conidial germination of *B. theobromae* was completely inhibited on ginger extracts at all concentrations.

It was observed that the growth of *B. theobromae* remained totally unaffected when the cultured fungus was exposed to smokes of rice straw, tobacco leaf and dhup for 5 to 15 minutes.

Mycelial growth of *B. theobromae* was completely inhibited on ginger extracts in combination with cowdung medium (20% ginger +15% cowdung) after four days of incubation at 28±2°C. There was no inhibitory effect on neem extracts (20% neem +15% cowdung) against this fungus.

Chapter 7

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