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# Effect of Temperature, Light and Media on Growth, Sporulation, Formation of Pigments and Pycnidia of *Botryodiplodia theobromae* Pat

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**Abstract:** *Botryodiplodia theobromae* Pat., is the causal organism of crown rot disease of banana. This fungus grew and sporulated at 10-40 °C, the optimum being 25-30 °C and the highest mycelial growth (78-90 mm) and sporulation (27-38 conidia/0.01 ml) were observed on PDA. There was no growth of the organism at 10 and 45 °C. Light was not necessary for growth, but it enhanced the sporulation. Maximum pigment formation occurred on PDA (Black 75% and White 25%) and minimum on PCM (Black 10% and White 90%). Formation of pycnidia initiated on 6th day in all the media, except Sabouraud's medium. Maximum pycnidia formation occurred on Czapek's (35/plate) medium and minimum on PCM (6/plate) medium. In case of glucose added PA medium, maximum pigment formation occurred on 35 gm of glucose added PA medium and minimum on PA medium. The highest mycelial growth was observed on PA medium (168 mm) and the lowest (54 mm) on 35 gm of glucose added PA medium. The highest number of pycnidia formed on PA medium (38/plate) and the lowest on 30gm glucose added PA medium (3/plate). There was no pycnidia formed on 35 gm glucose added PA medium.

**Key words:** Light, growth, sporulation, formation of pigment and pycnidia, *Botryodiplodia theobromae*.

## Introduction

*Botryodiplodia theobromae* Pat. is a well known parasite causing both field and storage disease of different crops, fruits and plantation trees (Khurana and Sing, 1972; Talukdar, 1974; Sing *et al.*, 1977 and Ilag and Marfil, 1977). It is an important pathogen of mango and other tropical fruits (Alam and Nahar, 1990) and causes black-band disease of jute, crown rot diseases of banana fruit, fruit rot of coconut fruit, stem-end rot of mango fruit, soft rot of papaw, guava, litchi, sapodilla fruit and die-back in lemon plants. Fungi exhibit varying response to light, depending on the light intensity, quality, and duration of exposure and temperature. Exposure to light is needed by some fungi for sporulation (Marsh *et al.*, 1959), whereas other fungi sporulate better in dark (Shoemaker, 1955) and with the decrease in germination of conidia as the period of darkness increased (Rewal and Grewal, 1989). Behaviour of a fungus/pathogen depends upon its nutritional response. Phytopathogenic organisms express a similarity in broader behaviour for their basic nutritional needs, yet they maintain their individuality for the choice of specific substances (Cochrane, 1958). It is now well established that phytopathogens show greater diversities in their ability to utilize the same elements from different nutrient media (natural, semi-synthetic and synthetic culture media). These culture media always contain essential elements needed for proper growth and sporulation of the organisms. Experiments were, therefore, conducted to determine the effect of temperature and duration of light on the growth and sporulation of *B. theobromae* on artificial media and effect of different media as these factors contribute in formulating the control measure for this organism and consequently in important diseases of fruit crops caused by it in different countries.

## Materials and Methods

The experiment was carried out during May' 2000 - March' 2001 in Department of Botany, Rajshahi University, Bangladesh. *B. theobromae* was isolated from diseased banana (*Musa sepientum* L.) fruit and culture was maintained on PDA plates. Five mm culture discs were cut with a sterile cork borer from advancing margin of colonies and kept on PDA plates and incubated at 10, 15, 20, 25, 30, 35, 40 and 45 °C. In case the study of light, carbon paper was used to wrap the petri dishes for darkness. Fluorescent lamp was used for light treatment and incubated under fluorescent lamp (one ft away) were subjected to light exposure as follows: (I) Continuous light; (ii) 16 hours light and 8 hours darkness; (iii) 8 hours light and 16 hours dark (iv) alternating light and darkness (12 hours); (v) alternating light and darkness (24 hours), and (vi) room conditions.

Potato Dextrose Agar (PDA), Richard's, PCM (paper chromatographs medium), Czapek's and Sabouraud's medium were used for the study of mycelial pigments and pycnidia formation. Different concentrations (0, 5, 10, 15, 20, 25, 30 and 35 gm) of glucose on PA (Potato-agar) medium also used for the observation of mycelial pigments and pycnidia formation, the following method was followed. Twenty ml of the media were poured after sterilization on 90 mm petri dishes. Five mm mycelial blocks from 7 days old culture of *B. theobromae* was inoculated on the center of media. The plates were incubated at 26 ± 2 °C for 15 days (Rewal and Grewal, 1989).

Colony diameter was measured in mm as basis of growth. Growth of the cultures was measured after three days and sporulation after fifteen days of incubation under various conditions and hemacytometer was used for measuring the degree of sporulation. The observation was made on the formation of pigment and pycnidia.

## Results and Discussion

**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 1. It was observed that the temperature range of 25-30 °C was optimum for mycelial growth (78-90 mm) of *B. theobromae* on PDA. But rest of the temperature had intermediary effect. The relative abundance of the mycelia increased with the increase in temperature. There was no mycelial growth and sporulation at temperature 10 and 45 °C. Sporulation occurred at 15-40 °C. The highest sporulation occurred at 30 °C (38 conidia/0.01 ml) and the lowest was at 15 °C (6 conidia/0.01 ml). Statistical analysis showed that with the increase in colony diameter, sporulation also increased, which is highly significant ($r = 0.951^{**}$ at 1% level).

Table 1: Effect of temperature on growth and sporulation of *B. theobromae* on PDA medium recorded after three and fifteen days of incubation.

| Temperature (°C) | Colony diameter (mm) | Sporulation (Conidia/0.01 ml) |
|---|---|---|
| 10 | 5[b] | 0 |
| 15 | 12 | 6 |
| 20 | 58 | 21 |
| 25 | 78 | 27 |
| 30 | 90 | 38 |
| 35 | 71 | 23 |
| 40 | 16 | 15 |
| 45 | 5[b] | 0 |
| $LSD_{(0.05)}$ | 1.9245 | 1.6098 |

$r_{(0.01)}$ = 0.951** 5[b] = 5 mm mycelial block
Average of three replications

These findings are similar with the observations of Quimio (1973), who studied the fungus *Colletotrichum gleosporioides* Penz. on artificial media.

**Effect of light on growth and sporulation:** *B. theobromae* was incubated under different light exposures for the observation of growth and sporulation after three and fifteen days on PDA (Table 2). The growth of *B. theobromae* was not affected much by different light conditions on PDA. *B. theobromae* sporulated in all the conditions, and the growth and sporulation occurred, while it was the highest in continuous light (90 mm and 112 conidia/0.01 ml). The lowest sporulation occurred in continuous darkness (24 conidia/0.01 ml) and by that time growth was measured to be 78 mm. It is evident from Table 2 that sporulation was fairly good (102 conidia/0.01 ml) in alternating light and darkness 12 hours and growth was measured to be 48 mm after fifteen and three days of incubation. Rest of the light conditions viz, 16 hours light and 8 hours darkness, 8 hours light and 16 hours darkness, alternating light and darkness (24 hours) (42 mm and 48 conidia/0.01 ml, 36 mm and 66 conidia/0.01 ml and 40 mm and 94 conidia/0.01 ml respectively) has intermediary effect in comparison with room conditions (84 mm and 72 conidia/0.01 ml). Statistical analysis shows that the presence of light has no significant relationship between colony diameter and sporulation (r =0.483 at 1% level). Rewal and Grewal (1989) studied the effect of light on conidial germination of three strains of *Botrytis cinerea,* infecting chickpea. They found that conidia of strain $B_1$ and $B_5$ germinated best under continuous light and strain $B_2$ germinated well under all the light/darkness treatments. From this study it is, therefore, revealed that light is not necessary for growth and sporulation of *B. theobromae,* but it enhances the growth and the number of conidia formation. It is in complete agreement with the observation of Ahmed (1985) and in partial agreement with the observation of Rewal and Grewal (1989).

**Effect of media on formation of pigments and pycnidia:** Formation of pigments and pycnidia of *B. theobromae* on different media were studied and results are presented in Table 3 and Fig. 1. Almost similar cultural characteristics were observed in all the media with slight variation in PCM. At the beginning, colour of the mycelia was white to light grey and became darker with the age. By the 5th day, colonies were olive brown and brown to black by the 9th day. Colonies were more or less regular in shape (circular). Results show that in all the media tested, two types of pigment formation were observed on culture plates. Maximum colour formation occurred on PDA (Black 75% and white 25%) and minimum on PCM (Black 10% and white 90%). Formation of pycnidia initiated on 6th day of incubation in all the media, except Sabouraud's medium, where pycnidia were evident only after 18 days. Pycnidia were often found partially embedded in the medium. They were visible from the reverse side of the petridish also. Results also showed that maximum pycnidia formation occurred on Czapek's medium (35/petridish) and minimum on PCM medium (6/petridish) and moderate types of pycnidia formation occurred on rest of the media after 15 days of incubation at 26±2 °C. There was no pycnidia formation on Sabouraud's medium. It is evident from Table 3 that size of the pycnidia are bigger when grown on PDA (3-5 × 2.0-3.80 mm) and Czapek's (2.10-5.10 × 1.90-3.25 mm) media than that of other media tested. Smaller pycnidia grown on PCM (1.0-3.25×0.50-2.15 mm). Quroshi and Meah (1991) studied the formation of pigments and pycnidia of *B. theobromae* isolated from mango stem-end rot on five different media. They observed that the highest number of complex pycnidia was formed on mango leaf extract agar (90/plate of 9 cm diameter) followed by PDA (36/plate) and found pink coloured pigment. Maximum pigmentation was observed in PDA (30-75% of the total mycelial surface) and moderate (15-25%) in PSA (potato sucrose agar). Pink colour development was low (5-10%) in Richard's agar and Czapek's agar and there was no such pigmentation on MLE-PDA (PDA supplemented with Crude extract of young mango leaves added before autoclaving at the rate of 50 ml/litre (MLE-PDA). The present findings are in partial agreement with those of Quroshi and Meah (1991). They observed pink pigment but our finding was black pigment and the highest pigment formation occurred in both cases of PDA. In case of pycnidia formation it differed. In present observation, the maximum pycnidia formation observed on Czapek's medium (35/petridish) and minimum on PCM medium (6/petridish). On the other hand Quroshi and Meah (1991) observed maximum pycnidia on MLE-PDA (90/plate) followed by PDA (36/plate).

Fig. 1: Effect of different media on formation of pigment and pycnidia after fifteen days of incubation. A=PDA, B=Richard's, C= Scabouraud's, D= Czapeak's, E=PCM medium.

Fig. 2: Effect of different concentrations of glucose on potato-agar (PA) medium for the formation of pycnidia, mycelial growth and pigmentation. A = 0gm, B = 5gm, C = 10gm D = 15gm, E = 20gm, F = 25gm, G = 30gm, H = 35gm glucose added on 1000ml PA medium.

Table 2: Effect of light on the growth of *B. theobromae* on PDA medium recorded after three and fifteen days of incubation.

| Treatments | Colony diameter (mm) | Sporulation (Conidia/0.01ml) |
|---|---|---|
| Continuous light | 90 | 112 |
| 16 hours light and 8 hours darkness | 42 | 48 |
| 8 hours light and 16 hours darkness | 36 | 66 |
| Alternating light and darkness (12 hours) | 48 | 102 |
| Alternating light and darkness (24 hours) | 40 | 94 |
| Continuous darkness | 78 | 24 |
| Room condition | 84 | 72 |
| $LSD_{(0.05)}$ | 3.2030 | 3.3862 |

$r_{(0.01)}$ = 0.483 Average of three replications

Table 3: Effect of different media for the formation of pycnidia and pigmentation of *B. theobromae* after 15 days of incubation at 26 ± 2°C.

| Media | Pigmentation | Number of pycnidia/ petridish | Length & breadth of pycnidia (in mm) | |
|---|---|---|---|---|
| | | | Length | Breadth |
| PDA | White-25% | | | |
| | Black-75% | 21* | 5-3 | 3.80-2.0 |
| Richard's | White-70% | | | |
| | Black-30% | 17* | 4.90-1.50 | 3.80-1.0 |
| PCM | White-90% | | | |
| | Black-10% | 6* | 3.25-1.0 | 2.15-0.50 |
| Czapek's | White-60% | | | |
| | Black-40% | 35* | 5.10-2.10 | 3.25-1.90 |
| Sabouraud's | White-30% | | | |
| | Black-70% | 00** | 00 | 00 |

$LSD_{(0.05)}$ 2.3099 * = Pycnidia formed after 6th days of incubations.
** = Pycnidia not formed after 15 days of incubation. Average of three replications

**Effect of different concentrations of glucose in Potato-agar (PA) medium on the formation of pycnidia, mycelial growth and pigmentation:** Effect of different concentrations of glucose in Potato-agar (PA) medium was tested for the formation of pigment, pycnidia and mycelial growth of *B. theobromae* after 15 days of incubation and results are presented in Table 4 and Fig. 2. Maximum pigment formation was observed on 35gm of glucose added medium (100% black) and minimum on PA medium (White, 95% and Black, 5%). It is observed from Table 4 that mycelial growth was faster on PA medium (168mm) and slower on 35 gm of glucose added PA medium (54mm). Pigmentation of the fungus increased with the increase in glucose of the medium (PA), but growth rate gradually decreased (168, 120, 76, 74, 72, 64, 60 and 54 mm) with the increase in glucose (0, 5, 10, 15, 20, 25, 30 and 35g). The highest pycnidia was formed on PA medium (38/plates) and the lowest on 30 gm of glucose added PA medium (3/plates), after 15 days of incubation at 26 ± 2°C. No pycnidia was formed on 35 gm of glucose added PA medium. Statistical analysis shows that with the increasing glucose in the medium, formation of pycnidia and colony diameter was decreased, which is negative correlation and highly significant ($r = -0.99^{**}$ and $-0.85^{**}$ at 1% level) and also show positive correlation and highly significant in case of colony diameter and pycnidia formation ($r = 0.90^{**}$ at 1% level). Osman *et al.* (1992) studied the effect of various culture conditions on *Alternaria alternata* and *Fusarium oxysporum* and measured growth, biomass and total lipid production. The best culture media were Czapek-Dox agar (MI) and Waksman's medium ($M_3$) for *A. alternata*. Optimum

Table 4: Effect of different concentrations of glucose in PA medium for the formation of pigment, mycelial growth and pycnidia of *B. theobromae* after 15 days of incubation at 26 ± 2°C.

| Concentration of glucose in PA (g) | Number of pycnidia/plate | Mycelial growth in petridish (mm) | Percentage of pigmentation |
|---|---|---|---|
| 0 | 38 | 168 | White-95<br>Black-5 |
| 5 | 32 | 120 | White-85<br>Black-15 |
| 10 | 23 | 76 | White-70<br>Black-30 |
| 15 | 19 | 74 | White-25<br>Black-75 |
| 20 | 13 | 72 | White-20<br>Black-80 |
| 25 | 9 | 64 | White-10<br>Black-90 |
| 30 | 3 | 60 | White-4<br>Black-96 |
| 35 | 0 | 54 | White-0<br>Black-100 |
| $LSD_{(0.05)}$ | 3.4737 | 6.4172 | |
| $r_{(0.01)}$ = 0.900** | | | |
| -0.99** | | | |
| -0.85** | | | |

Average of three replications

conditions were achieved by incubating cultures for 8 days at 30°C using sucrose as the carbon source. In *F. oxysporum*, MI and $M_5$ (potato dextrose agar) were the best culture media. Maximum lipid production was observed in culture grown for 10 days and incubated at 30°C using MI medium containing lactose as a carbon source. In present experiment, the growth was faster on PA medium than that of different concentrations of glucose added PA media. It was found that growth of *B. theobromae* was faster on PA medium and mycelium formation was thin. On the other hand, different concentrations of PA added glucose medium, the mycelial colony was thick, compact and growth rate was slower than PA medium with the development of aerial mycelium in colony and formed more pigment. Osman *et al.* (1992) observed that growth rate of both the fungi was faster on sucrose and glucose containing media and produced maximum lipid. The present findings are in partial agreement with the observations of Osman *et al.* (1992).

Most probably, with the increasing glucose in medium, the fungus could utilize it in a certain level and grow properly, and after that level, the fungal physiology does not permit the utilization of glucose for growth of the pathogen. There the fungus might utilize the glucose by different ways instead of growth and formed more pigmentation using more glucose.

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