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EFFECT OF MEDIUM, TEMPERATURE AND pH, ON *IN-VITRO* GROWTH OF *Botryodiplodia theobromae* ISOLATED FROM GUAVA

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Abstract

Botryodiplodia theobromae is a major reason for bringing huge economic losses to the crop production in tropical and subtropical regions. In this study outcomes of media, temperature and pH were evaluated on mycelial colony growth of the fungus. Potato Dextrose Agar (PDA) and Czepak Dox Agar (CDA) were suitable for the growth of fungus colony. The highest growth was seen on PDA. The fungus grew best at 25-30°C. The fastest growth was observed at 30°C. There was no growth at 10°C. pH 7 and 8 were the most optimum for the fungus growth whereas, the minimum growth was obtained at pH 4.

Key word: *Botryodiplodia theobromae*, media, temperature, pH

Introduction

Botryodiplodia theobromae (Pat.) Griff. and Maubl. (Syn: *Lasiodiplidia theobromae* Pat.) and the asexual conditions of *Botryosphaeria rhodina* (Berk and M.A. Curtis) Arx are the fungi, having enormous importance economically. The fungus *B. theobromae* is a kind of an opportunistic pathogen which brings various kinds of diseases such as tuber rots in yam and sweet potatoes and root rot in cassava. It as well causes banana crown rot, peanut collar rot, mango stem end rot, pawpaw stem rot and citrus leaf spot (Rossel et al., 2008, Khanzada et al., 2004, Jiskani, 2002, Arjunan, 1999, Sangchote, 1988). It has been widely spread worldwide in hot and semi-hot regions (Faber *et al.,* 2007). The host range of this pathogen is approximately there are above 280 species of plants on which this pathogen invades (Domsch *et al.,* 2007, Khanzada *et al.,* 2006, Sutton, 1980). Guava decline is one of the most serious diseases of guava that brings considerable losses to its production, is caused by *B. theobromae;* this is a predominant pathogen which is mainly responsible for decline (Bokhari *et al.,* 2008). *B. theobromae* has been recognized by Jiskani (2002) and Sangchote (1988) as one of the common isolates and a virulent pathogen on infected mango fruits in Pakistan. However, it is not a contradictory problem nowadays because *B. theobromae* is considered as the main factor for decline in many regions (Al-Adawi *et al*., 2003, Iqbal *et al.,* 2004, Malik *et al.,* 2005 and Al-Adawi *et al.,* 2006). Invasion of pathogen is reported to increase by the beetle (*Xyleborus affinis*), relative humidity 80% and in optimum temperature (25-31°C) (Rawal, 1998). The pathogen first starts to rot the bark at one point then progresses and finally engulfs the whole stem leading to the plant death (Malik *et al.,* 2003). Growers are perturbed over this fatal disease as its incidence in some areas is 7.51 % (Iqbal *et al.,* 2007).

The target of the current study was to evaluate the effects of different media, pH levels and ranges of temperatures on the mycelial colony growth of *Botryodiplodia theobromae* obtained from the diseased samples of guava.

MATERIAL AND METHODS

The experiment was carried out during the year 2016 in Department of Plant Pathology, Sindh Agriculture University Tandojam.

Isolation, identification and purification of the pathogen: Isolation of fungi was carried out by standard procedure of isolation. The collected diseased specimens were carefully washed, and then cut into desirable pieces about 3 to 5mm including some healthy section and surface sterilized with 5% sodium hypochlorite for five minutes. These pieces were placed on PDA and then incubated at 28°C for 7 days. The observations for the fungal growth were monitored daily. The fungi that grew on these pieces were further purified and identified with respect to their morphological characteristics (Pitt and Hocking, 2009).

Effect of different media: Effect of five different media *viz.,* Potato Dextrose Agar (PDA), Vegetable-8 Agar (V-8), Czapek Dox Agar (CDA), Nutrient Agar (NA) and Guava Leaf Extract Agar (GLEA) were assessed on the mycelial colony

growth of *B. theobromae*. Five mm disk of seven days old culture of *B. theobromae* was with a hygienic cork borer from the colonies and placed in the center of Petri plate containing autoclaved media and incubated at 28°C temperature. Each medium had 3 replicated plates. The dimension of colony in every plate was noted, at 24 hours interval for seven days.

Effect of different temperature ranges: Effect of various temperature ranges for the maximum mycelial colony growth of *B. theobromae* was evaluated. Five mm disk of seven days old culture of *B. theobromae* was detached with a hygienic cork borer from its colonies and placed in the middle of Petri plate containing autoclaved PDA medium and each treatment containing three replications was incubated at 10°C, 15°C, 20°C, 25°C and 30°C for seven days. The colony diameter was noted on daily basis.

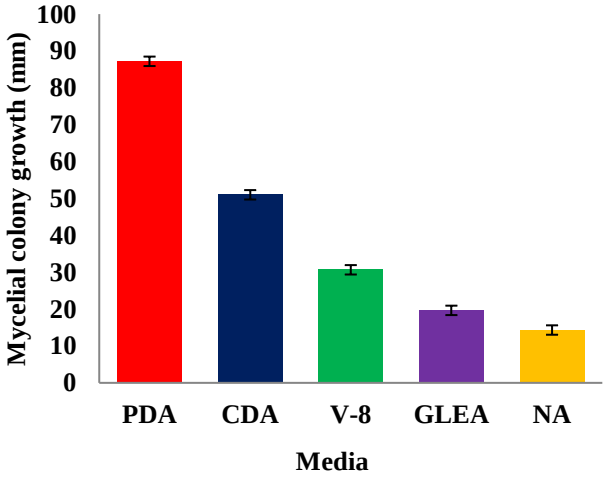
Effect of different pH levels: Effect of different pH levels on the mycelial colony growth of *B. theobromae* was evaluated. To get the desired pH levels of 4.0, 5.0, 6.0, 7.0 and 8.0, the pH of PDA medium was maintained by adding either hydrochloric acid (0.1M HCl) or sodium hydroxide (0.1M NaOH). An electrical pH meter was used to adjust the pH of the media prior to sterilizing in the autoclave at 121ºC. The fungus cultures were placed in petri dishes which were already with PDA and the plates were kept in incubator at 28°C for seven days. The finest pH for the colony growth of the fungus was determined by calculating colony diameter on daily basis.

Data Analysis: The data were recorded by measuring the growth of *Botryodiplodia theobromae* in every treatment .The results obtained were analyzed through Students Statistical Software Package (Statistic ver. 8.1) to have the significant differences in mycelial colony growth of *B. theobromae* among the all treatments.

RESULTS AND DISCUSSIONS

Isolation and identification of *Botryodiplodia theobromae:* The fungus *Botryodiplodia theorbomae*, isolated from infected parts of guava tree was identified on the basis of the morphological characteristics and colors of the colony of fungi in accordance with the studies of Pitt and Hocking, 2009; Khanzada *et al.*, 2006. The colony characteristics were as well in confirmation with the studies of Philips (2007), where he studied that *B. theobromae* colonies were often grayish sepia to mouse grey to black, fluffy with abundant aerial mycelium. The matured cultures were having black pigmentation. The results were also linked with the studies of Godfried (2012), who compared 25 isolates on PDA at 28°C, and all the isolates in the culture developed black pigments after 48 hours of inoculation.

Effect of different media on mycelial colony growth of *B. theobromae:* The radial growth rates of mycelia of *B. theobromae* were notably affected by different media (Figure 1). Initially the mycelia color was white to light grey and gradually turned to darker color on all the media. The highest mycelial colony growth of the test fungus was observed on PDA medium followed by CDA medium showing 87.20 mm and 51.00 mm colony diameter respectively. On V-8 and GLEA media intermediate mycelial growth was observed, showing 30.66 mm and 19.66 mm colony diameter respectively, whereas the minimum mycelial colony growth was seen on NA medium, showing 14.33 mm colony diameter (Fig.1). Our observations were with conformity to the results of Alam *et al.* (2001), where he observed the maximum *B. theobromae* mycelium growth on Potato Dextrose Agar and on Czapek Dox agar medium. Likewise, Qureshi & Meah (1991) recorded the fastest mycelial growth of this fungus on Potato Dextrose Agar.



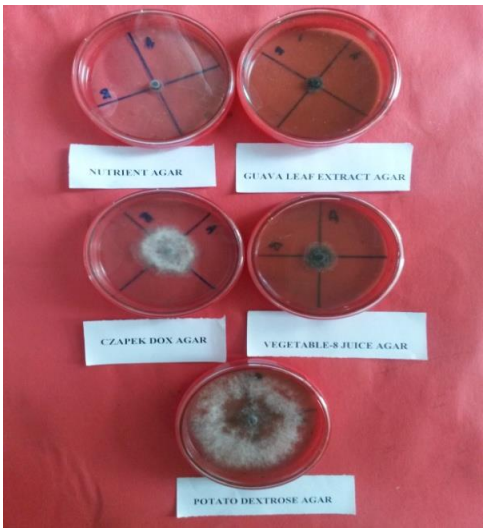
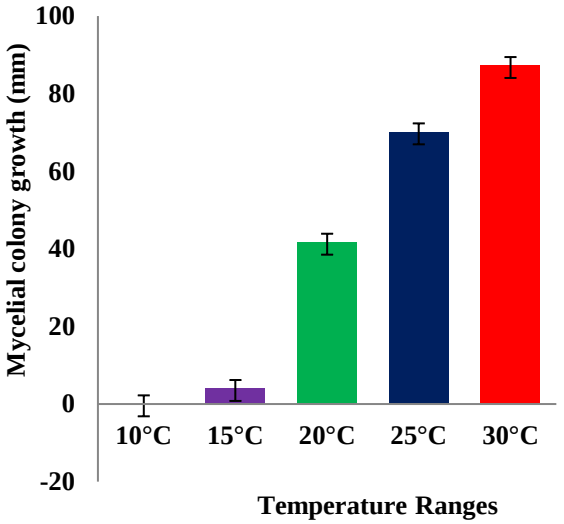
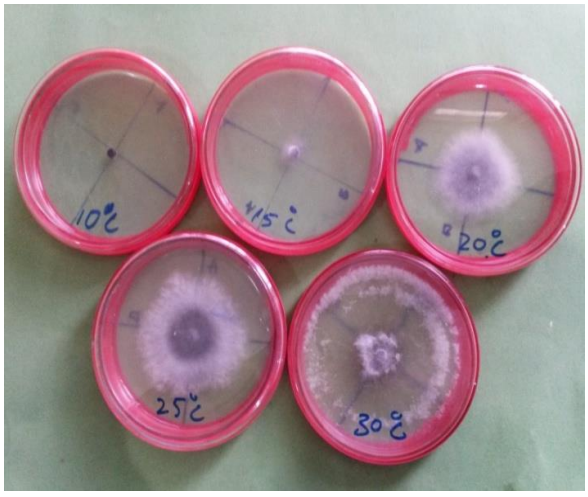


Figure: 1. Effect of different media on the mycelial growth of *B. theobromae*

Effect of different ranges of temperatures on mycelial growth of *B. theobromae:* The colony growth of *B. theobromae* in response to adjustments in temperature proved variability on PDA medium (Figure 2). It was observed that the temperature ranges of 25°C mm and 30°C were optimum for the fastest mycelial of the fungus, showing 70.10 mm and 87.20 mm colony diameter respectively. Other temperature ranges had moderate effect. The mycelial growth rose up with the increased in temperature. The intermediate mycelial growth was observed at 20°C, showing 41.66 mm colony diameter. The lowest growth was observed at 15°C showing 3.96 mm colony diameter. There was no mycelial growth observed at 10°C (Fig.2). Our studies are in accord with the observations of Alam *et al.* (2001), who observed that the temperatures ranging from 25 to 30ºC were most favorable for the mycelial growth of *B. theobromae*, though rest of the temperature had intermediary effect. There was no growth of mycelium at 10 and 45 ºC. Rehman *et al.* (2011) observed the highest growth of *B. theobromae* when it was incubated at 30 ºC and 25 ºC, and the minimum growth was obtained when *B. theobromae* was incubated at 15 ºC. Khanzada *et al*. (2006) studied that the fungus was capable of growing at temperatures from 20 to 45ºC. The fungus growth was highest at 30 to 40ºC with no any colonial growth of fungus below temperature 15ºC.





Picture

Figure: 2. Effect of different ranges of temperature on mycelial colony growth of *B. theobromae*

Effect of different pH levels on the mycelial colony growth of *B. theobromae:* The pH levels from 4 to 8 significantly affected the mycelial colony growth of *B. theobromae* (Fiure.3). The highest mycelial colony growth was observed at pH 7 followed by pH 8, showing 88.00 mm and 87.06 mm colony diameter respectively. Relatively less growth was obtained at pH 6 (54.66 mm), while the minimum growth was seen at pH 5 followed by pH 4, showing 33.66 mm and 21.33 mm colony diameter respectively. The data analysis showed that there were no significant differences at 7 and 8 pH levels (Fig.3). Our observations noticeably show that the optimum pH levels for the myc-

elial colony growth of *B. theobromae* are 7 and 8 pH. Our studies have similarities with the records of Rehman *et al*., (2011) who recorded the maximum mycelial growth of *B. theobromae* on media in which pH levels were adjusted at 7 and 8 pH, and relatively less growth was recorded on media in which pH was adjusted at pH 6 and minimum growth was obtained at pH 4. Patil and Pathak (1993) also studied that the fungus grew best on pH level of 7 and 8.

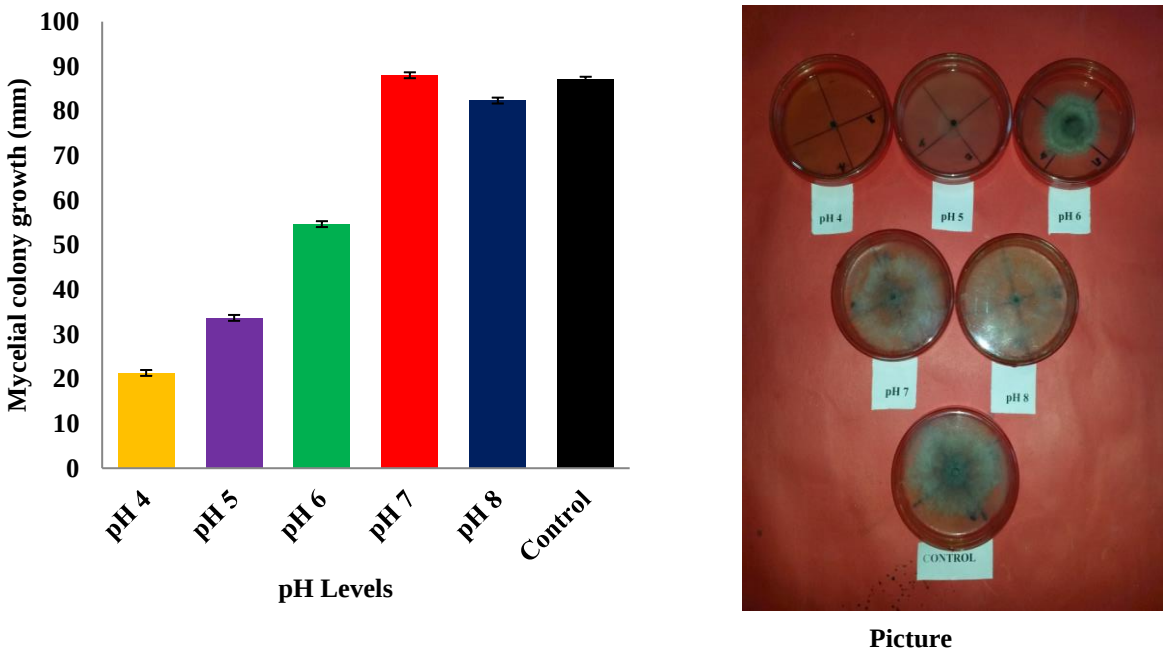


Figure 3: Effect of different pH levels on mycelial colony growth of *B. theobromae*

Conclusion

We conclude that the *Botryodiplodia theobromae* can normally grow on various media, temperature and pH levels, our findings demonstrate that PDA medium, temperature range at 30 ºC and pH level of 7 are the most optimum approaches for the growth of the *B. theobromae* which will help the researchers to carry out more extensive studies on this fungus keeping the current studies in view.

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