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# EFFECT OF CULTURE MEDIA ON GROWTH, COLONY CHARACTER AND SPORULATION OF THREE FOLIAR PATHOGENS OF GUAVA

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**ABSTRACT**
The mycelial growth rate (radial and biomass), colony character and sporulation pattern of three folier pathogens of guava viz., *Pestalotiapsidii, Colletotrichum gloesporides, Botriodiplodia theobromae*) were observed on six different culture media after seven days of incubation at 25 ± 2ºC. The six different culture media used were namely, Potato Dextrose (PDA), Czapek'sDox (CD), Richard's (RM), Oat meal (OMA), Mature and Immature Guava Fruit extract Agar medium. The culture growth, characteristics (texture, surface, reverse colouration and zonation) and sporulation of three test fungi were significantly influenced by the type of medium used. Richard's medium exhibited comparatively higher mycelial growth and sporulation for all the three test fungi, except *P. psidii* which preferred to grow on PDA in respective parameters. The poor performance of the three pathogens was recorded on Oatmeal and guava fruit extract media.

## INTRODUCTION

Guava (*Psidium guajava* L.) is an important fruit crop of India and is considered as poor men's apple. Its production is low in India because of several important diseases causing pathogens like *Pestalotiapsidi, Colletotrichum gloesporides and Botriodiplodia theobromae* is one of them by which it suffers. Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. Growth and sporulation are an important phase during the life of fungi as it determines their continuance from one generation to the other. Easy growth, sporulation, destination and capacity of resistant against unfavourable conditions are some of the distinct advantages of the spores which help in propagation. Growth and sporulation of fungi is considerably influenced by external and as well as internal conditions also. Among the external factors nutrition's is one of them which werepreviously proved in same genera of respective pathogen by several workers (Ramaiah and Seshadri, 1980; Hiremath *et al.,* 1993; Ekboto *et al.,* 1997; Pati *et al.,* 2001; Awasthi, 2003; Randhwa and Munshi, 2003; Rani and Murthy, 2004; Kasar, 2004; Younis *et al.*, 2004) by use of Different media. Considering the prevalence and significance of these three foliar guava diseases and importance of the crop, present investigation was under taken to determine the best medium and optimal growth conditions of the pathogen *in vitro* to establish the suitability of media for the mycelia growth and sporulation of the fungal pathogens.

In the present study the effect of culture media on the growth and sporulation of three folar pathogens of guava were studied.

## MATERIALS AND METHODS

To study the effect E of culture media on growth and sporulation of respective pathogen, were studied by using the method described by (Sharma and Pandey, 2010). The vegetative growth was evaluated by measuring the colony diameter as well as colony characters through visual observation. The diametric growth (mm) was measured at right angles at interval of 24 hours upto 7 days. The growth rate (rg) in mm/h was calculated on each medium by the following formula by Randhwa and Munshi (2003).

Measurement of radial growth over the control (PDA) was done by calculating the per cent increase / decrease of radial growth of respective pathogen in different media against PDA medium by following formula.

Per cent increase / decrees of radial growth over PDA

$$= \frac{Td - Ti}{Td} \times 100$$

Where,

Td = Radial growth (mm) in PDA

Ti = Radial growth (mm) in other media.

Growth character of the respective pathogen was studied at 24 hours interval with respect of amount of aerial mycelium, texture of mycelium, development of pigmentation of mycelium, appearance of fruiting bodies and their aggregation. The efficacy of different media on spore

production was determined by counting the number of spores per ml of spore suspension using haemocytometer. Five mycelial discs of 5.0 mm diameter were scooped out by sterilized cork borer from young growing regions of colony. The discs were suspended in 10 ml sterilized distilled water and shaked gently with the help of wrist action shaker with the addition of a Teepol to ensure complete detachment of spore from mycelial plugs. The population of spore per ml. was subsequently determined with the help of haemocytometer.

For liquid medium, in 150 ml conical flasks, each with 50 ml broth, was inoculated with a 5.0 mm mycelial plug as before. The inoculated flasks were incubated at 28 + 1oC for 10 days in a BOD incubator. The dry weight of mycelial mat of each flask was harvested by filtering through folds of pre-dried and weighed filter papers, rinsed thoroughly and preserved in dessicater over P2O5 for overnight. The final weight of dried mycelial mat was recorded after drying of mycelial mat in an oven at 60oC for 24 hrs.

To study the per cent increase or decrease of bio-mass of respective pathogen in different media over the control (PDA) was determined using formula as below:

% increase or decrease of biomass of pathogen over PDB = Wd - Wi / Wd x 100

Where, Wd = Biomass from PDB medium

Wi = Biomass from other broth medium

The spore population per ml was recorded with help of haemocytometer in which, 1.0 ml homogenized suspension of the culture filtrate was taken from each flask separately and diluted in 5.0 ml sterilized distill water from which 0.1 ml suspension was taken on a slide and average number of conidia present in four microscopic fields (150×) were counted.

## RESULTS AND DISCUSSION

Six different synthetic and semi-synthetic media were used to study the effects on growth, sporulation and growth rate of test pathogens. The maximum growth and sporulation were recorded in Richard's synthetic medium by *B. theobromae* and *C. gloeosporioides* followed by Potato dextrose and Czapek'sdox media. The poor growth without sporulation was recorded in Oatmeal and mature and immature guava fruit extract media by same pathogens. In case of *P. psidii*, the good response was obtained in Potato dextrose medium followed by Richard's, Czapek'sdox and Oatmeal, respectively (Table 1-3). The poor growth without sporulation was obtained in guava fruit extract medium. The growth rate/hr of respective pathogen was recorded in same medium for a fixed time of incubation and the maximum growth rate was recorded in PDA medium for *P. psidii*, *C. gloeosporioides* and *B. theobromae* at 72 and 48 hrs followed by RSA, CDA, OMA and GFEA, respectively (Fig. 1-3).

Present study it was concluded that the culture media also influenced the growth and sporulation of all three pathogens. It was previously proved in same genera of respective pathogen by several workers (Ramaiah and Seshadri, 1980; Younis*et al.*, 2004 and Kasar, 2004 in *Pestalotia* sp., Hiremath*et al.*, 1993; Ekboto *et al.*, 1997; Randhwa and Munshi, 2003; Awasthi, 2003, Rani and Murthy, 2004 in *Colletotrichum*sp.

**Table 1: Effect of culture media on growth and sporulation of P. psidii**

| Medium | Mean value* | | | | | Cultural characteristics |
|---|---|---|---|---|---|---|
| | Colony diameter (mm) | Increase/ decrease in growth over PDA (%) | Dry mycelial wt. (mg) | Increase/ decrease in growth over PDA (%) | Sporulation (x 105 / ml ss) | |
| Czapek'sdox | 78.8 | - 12.4 | 383.5 | 18.0 | 39.5 | Thick, white mycelial growth without concentric rings; sporulation started from fifteen days after inocubation. Very big size black dot spore masses were observed appeared very less on the medium. |
| Richard's synthetic | 82.4 | - 8.4 | 402.8 | 13.8 | 45.0 | Very Thick, white mycelial growth without concentric rings. Sporulation occurred on the tenth day after inocubation, small black dot like spore masses were observed, scattered on the medium |
| Oat meal | 46.8 | - 48.0 | 267.5 | 42.8 | 18.4 | Thin, off white mycelial growth without concentric rings, initial sporulation observed on seventeen days of inoculation. But very less sporulation scattered on the medium. |
| Mature guava fruit extract | 36.0 | - 60.0 | 236.5 | 49.4 | - | Very, thin, off white cottony mycelial growth without concentric rings, sporulation completely absent. |
| Immature guava fruit extract | 28.4 | - 68.4 | 127.5 | 72.7 | - | Poor, off white, cottony growth, without concentric rings and sporulation. |
| Potato dextrose | 90.0 | - | 467.5 | - | 51.0 | Very thick, white, mycelial growth arranged in concentric rings; sporulation started from seventh day after inoculation, heavy sporulation as black spore masses just beneath the mycelial net. |
| SEd (±) | 0.15 | - | 2.2 | - | - | |
| CD at 0.01% | 0.5 | - | 6.5 | - | - | |

*Mean value is the average of four replications.

**Table 2: Effect of culture media on growth and sporulation of *C. gloeosporioides*.**

| Medium | Mean value* | | | | | Cultural characteristics |
|---|---|---|---|---|---|---|
| | Colony diameter (mm) | Increase/ decrease in growth over PDA (%) | Dry mycelialwt (mg) | Increase/ decrease in growth over PDA(%) | Sporulation (x 105/ ml ss) | |
| Czapek'sdox | 72.3 | - 10.7 | 384.1 | - 4.5 | 34.0 | Good growth with smooth margin, mycelium at first white later becomes slight greyish black. Abundant sporulation started from twelve days of incubation. |
| Richard's synthetic | 89.4 | - 9.4 | 500.0 | + 18.8 | 49.2 | Very thick good growth with smooth margin, mycelium at first white later becomes greyish black. Abundant sporulation started from six days of incubation. |
| Oat meal | 47.0 | - 42.0 | 289.3 | - 28.8 | - | Poor growth, with smooth margin, mycelium at first white later becomes slight greyish black, no sporulation. |
| Mature guava fruit extract | 39.4 | - 51.3 | 260.0 | - 36.0 | - | Poor, off white cottony mycelial growth later become slight greyish black without sporulation. |
| Immature guava fruit extract | 30.2 | - 62.7 | 190.0 | - 53.2 | - | Very poor, off white cottony mycelial growth, become slight greyish black, no sporulation. |
| Potato dextrose | 81.0 | - | 406.2 | - | 40.6 | Very thick, good growth, margin irregular, mycelium at first white later become dark greyish black, acervulus formation started from seventh day of incubation and sporulation ten days onwards. |
| SEd (±) | 0.05 | - | 3.7 | - | - | |
| CD at 0.01% | 0.15 | - | 10.8 | - | - | |

*Mean value is the average of four replications.

**Table 3: Effect of culture media on growth and sporulation of *B. theobromae***

| Medium | Mean value* | | | | | Cultural characteristics |
|---|---|---|---|---|---|---|
| | Colony diameter (mm) | Increase/ decrease in growth over PDA (%) | Dry mycelialwt (mg) | Increase/ decrease in growth over PDA (%) | Sporulation (x 105 / ml ss) | |
| Czapek'sdox | 76.1 | - 11.7 | 361.6 | - 15.1 | 39.7 | Good, wooly, white mycelium later becomes slight pink in colour and sporulation started at ten days. |
| Richard's synthetic | 90.0 | + 42.2 | 525.8 | + 19.01 | 53.0 | Fine, wooly pinkish, white colour mycelia, sporulation started from seven days after incubation. |
| Oat meal | 54.0 | - 37.3 | 282.0 | - 33.8 | - | Poor, white, cottony, scattered growth and no sporulation. |
| Mature guava fruit extract | 45.3 | - 47.4 | 206.8 | - 51.5 | - | Very poor, off white, cottony, scattered growth without sporulation. |
| Immature guava fruit extract | 29.6 | - 65.7 | 170.0 | - 60.0 | - | Very poor, off white, cottony mycelium with scattered growth and no sporulation. |
| Potato dextrose | 85.2 | - | 425.8 | - | 44.1 | Very good, wooly, white, mycelial growth with sparse margins; aerial part of mycelium later become pinkies colour and basal portion become dark grey colour due to high pigmentation. Sporulation started from ten days after incubation. |
| SEd (±) | 0.1 | - | 2.6 | - | - | |
| Cd at 0.01% | 0.3 | - | 7.6 | - | - | |

*Mean value is the average of four replications.

and Pati *et al.*, 2001 and Awasthi, 2003 in *Botryodiplodia*sp). Richard's synthetic medium was found to be most suitable for growth and sporulation of all these pathogens except *P. psidii* that favoured Potato dextrose medium. Similar results have been reported by Hiremath *et al.* (1993), Ekoboto *et al.* (1997), Randhawa and Munshi (2003), Awasthi (2003), Rani and Murthy (2004) in *Collotetrichum*sp. and *Diplodia* sp. causing anthracnose and fruit rot diseases on different host plant. On the other hand Ramaiah and Seshadri (1980), Patil*et al.* (1988), Sinclair and Backman (1989), Aneja (1993) Dhingra and Sinclair (1995), Randhwa and Munshi (2003), Youni*set al.*, (2004) and Kasar (2004) observed that PDA (Potato dextrose agar) medium was most suitable for growth and sporulation of *Phomopsissojae, Pestalotia*sp.*Diplodia*sp. and some other pathogens. PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Maheshwari *et al.,* 1999; Saha *et al.,* 2008). Study with different culture media containing both carbons (C) and nitrogen (N) sources and their mechanism of specificity on growth and reproduction of fungi is now well understood. But it has been suggested that the primary basis is the concentration of C and higher concentration C and N induces vegetative

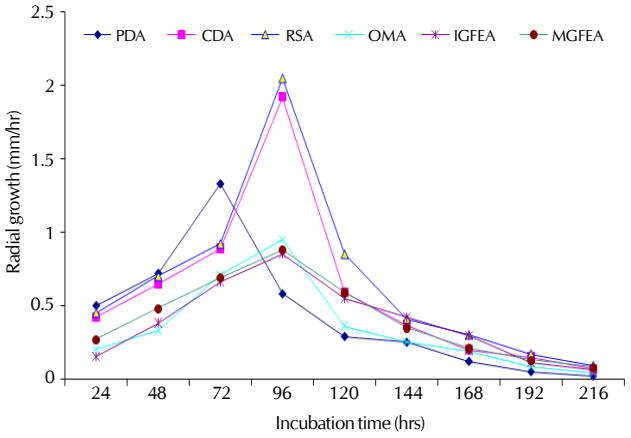


**Figure 1: Radial growth (mm/hrs) of *Pestalotiapsidii, Colletotrichumgloesporides, Botriodiplodiatheobromae*) on six different culture mediaviz., Potato Dextrose (PDA), Czapek'sDox (CD), Richard's (RM), Oat meal agar (OMA), Immature guava fruit extract agar (IGFEA) and Mature guava fruit extract agar (MGFEA).**

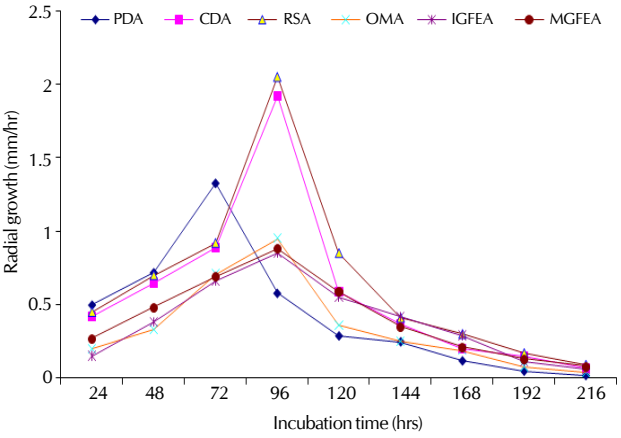


**Figure 2: Radial growth (mm/hrs) of *Colletotrichumgloesporides* on six different culture mediaviz., Potato Dextrose (PDA), Czapek'sDox (CD), Richard's (RM), Oat meal agar (OMA), Immature guava fruit extract agar (IGFEA) and Mature guava fruit extract agar (MGFEA)**

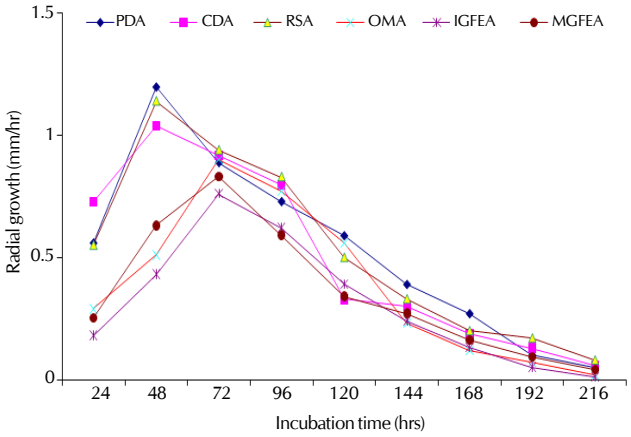


**Figure 3: Radial growth (mm/hrs) of *Botriodiplodiatheobromae* on six different culture mediaviz., Potato Dextrose (PDA), Czapek'sDox (CD), Richard's (RM), Oat meal agar (OMA), Immature guava fruit extract agar (IGFEA) and Mature guava fruit extract agar (MGFEA)**

growth (Hirsch, 1954). Abundant mycelial growth of three pathogens on culture media was, therefore, due to presence of both C and N sources.

Some workers reported the variable effects of different media in respect of variation in growth characters in respect of *Helminthosporium* sp. and *M. phaseolina*. Shafey et *al.* (1984) for example, studied the cultural and morphological characters on five different culture media. Raut and Bhome (1997), however, reported that Elliot's medium containing C and N sources was the best for the growth of *R. bataticola* (=*M. phaseolina*), while Singh and Chohan (1982) observed that Czapek's Dox was the best for mycelial growth of *M. phaseolina*. Eight different media including synthetic and semi synthetic in solid and liquid state were tested for their suitability to the growth and sclerotial formation of the fungus, *Macrophomina phaseolina* by Tandel et al. (2012). The growth of *M. phaseolina* was more significant on potato dextrose agar (89.67mm) as compared to other media used but was at par with potato carrot sucrose agar (86.67mm). The sclerotial formation of *M. phaseolina* was high on potato dextrose agar, potato carrot sucrose agar and Richard's agar. Among the various liquid media tested, significantly higher dry mycelial weight was yielded in Richards' solution (776.25mg) as compared to the rest of the liquid media but was at par with Czapek's Dox solution (758.68mg). Similarly, Lal *et al.* (2014) evaluated different media to determine optimal conditions for mycelial growth and sporulation of *Curvularia lunata* causing curvularia leaf spot of blackgram. *In vitro* studies found that amongst solid media, Potato dextrose agar (7.60 cm) and Host extract agar (6.73 cm) were the best for fungus growth and sporulation followed by Conn's agar and Czapek (Dox) agar. Whereas, in liquid media, Richard's (834.33mg), Czapek (Dox) (830.00mg), supported best growth of the fungus and sporulation.

The differences in mycelial growth of three pathogens on culture media were, therefore, due to presence of both C and N sources. Rani and Murthy (2004) concluded that the variations in growth of the fungus on various media may be attributed to the differences among the media in terms and availability of nutrients required by the fungus. So, from the results of the present study and also from the evidence produced here it may be concluded that the presence of C alone or in combination with N favoured the growth of three pathogens namely *P. psidii*, *C. gloeosporioides* and *B. theobromae*.

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