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# Effect of Culture Media, Temperature and pH on Growth and Sporulation of Alternaria Alternata Causing Blight of Castor

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**Abstract**

Maximum vegetative growth and best sporulation of pathogen was recorded on Kirchoff's agar medium at 25-30*C with optimum at 30 c and 6.5 pH in complete dark condition.

**Keywords:** Culture Media, Alternaria Alternata.

**Introduction**

Alternaria blight caused by Alternaria alternata is one of the diseases of castor plant causing defoliation, leaf falling, delayed fruiting and heavy losses in yield in India (34.4%). The investigations were carried out in vitro to determine the effect of culture media, temperature and pH on the growth and sporulation on Alternaria alternata.

**Objective of the Study**

Aims and Objective of present investigation is to assess the nutritional requirement and preferences of the pathogen Alternaria causing leaf blight in castor plant. The investigation will be of great interest for growing the pathogen over synthetic and artificial media in the laboratory for various research purposes.

**Material and Method**

The pathogen was isolated from infected castor plant and used during the period of entire study. Ten agar media viz, Kirchoff's P.D.A, Richard's, corn meal, Asthana and Hawker's, leaf decoction, oat meal, C.D.A, sabouraud's, and Brown's starch were used. 20 ml of the sterilized media was poured in each sterilized petridish and was incubated with 5mm discs of Ten days old culture of pathogen grown on 2% P.D.A. The inoculated petridishes were then incubated at 30*C. Observations on radial growth of fungus was recorded in mm in two directions at right angles to each other and the average was calculated after nine days to study the effect of temperature on growth and sporulation of pathogen, it was grown at eight different temperatures, viz 5,10,15,20,25,30,35 and 40*C in different incubators. In this study kirchoff's medium was used as the basal medium and pH was maintained at 6.5 taking 50 ml media in each conical flask incubated with 5mm mycelia disc of 10 days old culture of the fungus after inoculation for 10 days, dry mycelium weights were determined after filtering the mycelium mat on Whatman's filter paper1.Mycelia mat was thoroughly washed with distilled water and finally dried to a constant weight at 80*c for 48 hrs, subsequently cooled in desiccator and weighted on analytical balance. Average dry weight of replicates was taken as standard value for the comparison of growth under different treatments. The pH of filterate was measured with the help of digital electronic pH-meter. Similarly, twenty pH levels ranging from 2.0 to 11.5 were maintained on basal Kirchoff's medium. The pH of the medium was adjusted before autoclaving with the help of Hcl (0.1 N) and NaoH (0.1 N) using digital pH meter.

The spore suspension of Aalternata was diluted to make concentration of nearly 50-70 spores/low microscopic field. Fresh spore suspension was allowed to germinate on slides, which incubated at temperatures viz. 5,10,15,20,25,30,35and40*c and the germination was recorded after 10 and 18 hrs. For studying the effect of pH on spore germination, slides with the spore suspension of the fungus were placed at 84,87,90,93,95,97 and 100% artificially created relative humidity as per

method of Wilson (1921) in desiccator. Average percentage germination was calculated after 10 and 18 hrs.

**Results and Discussion**

The radial growth of pathogen was found to vary significantly with respect to different media (table1), the growth and sporulation of the fungus was significantly higher in kirchoff's agar medium (098.25mm). PDA (83.50) medium also supported better growth and sporulation but was inferior to Kirchoff's. Other media in descending order of merit were Richard's, corn meal, Asthana Hawker's, leaf decoction, oat meal, czapekdox, sabouraud's and Brown's medium supported poorest growth and sporulation (18.50mm). These results are in conformity with the results obtained by Ashour and El-kadi (1959).

**Table 1: - Average Radial Growth and Sporulation of Alternaria-alternata on Different Agar Media after 9 days of Incubation Period at 30+1*c.**

| Media | Average Dia. Of Colony(mm) | Average rate of growth (mm)per hour | Colonial growth | Nature of growth | Degree of Sporulation |
|---|---|---|---|---|---|
| Kirchoff's | 98.25 | 0.447 | Very good | Pale grey wavy, Zonation Present | ++++ |
| P.D. A | 83.50 | 0.37 | Very good | Dusty white, Circular, Zonation Absent | ++++ |
| Richard's | 77.25 | 0.366 | good | Smoky Grey, Circular, Zonation Present | ++++ |
| Corn-meal | 73.25 | 0.341 | good | Smoky Grey, Circular, Zonation Absent | +++ |
| Asthana's And Hawker's | 69.00 | 0.316 | good | Smoky Grey, Wavy, Zonation Absent | ++ |
| Leaf decoction | 68.75 | 0.291 | good | Pale smoky Grey, Circular, Zonation Absent | +++ |
| Oat meal | 68.75 | 0.283 | good | Pale smoky Grey, Circular, Zonation Absent | +++ |
| C.D. A | 54.25 | 0.270 | good | Pale grey, Circular, Zonation Absent | +++ |
| Sabouraud's | 24.50 | 0.145 | poor | Smoky Grey, Circular, Zonation Absent | ++ |
| Brown's Starch | 18.50 CD at 5%P-4.147 | 0.104 | poor | Smoky Grey, Circular, Zonation Absent | + |

**Legend-** nil (-), Poor (+), Moderate (++), Fair (+++), Profuse (++++).

In the present investigation better growth, a pathogen on synthetic medium (kirchoff's) than natural medium (PDA) may be due to presence of essential elements in synthetic medium in utilizable form. The variation however in the growth and sporulation of the pathogen over different synthetic media is attributed to the nature of elements and their concentration used in the media and also the compounds used in which these elements occur. Colonial growth of pathogen was found sub fluffy in most of the media used including kirchoff'smedium; fluffy in case of Richard's, Cornmeal, Czapek dox and Sabouraud's media. The shape of the colony was circular in almost all the media except in Kirchhoff's and Asthana Hawker's where it was wavy. Zonation was found only in Kirchoff's and Richard's media. The colour of mycelium was slightly different in different media. These results were conformatory with (Swank (1951) on A. alternata, Prabhu (1965) on A. triticina and Dhanraj (1970) on A. alternata. (Ionnidis et al (1973), Usha Rani et al (1984), Dutta et al (2003), Saha et al (2008), Munde et al (2013), Patil and Suryawanshi (2015)). Pigmentation could not be marked in any media. Average rate of radial growth of the pathogen per hour was highest (0.447mm) in Kirchhoff's agar medium, followed by PDA, Richard's, Cornmeal, AsthanaHawker's, leaf decoction, Oat meal, CDA and Sabouraud's agar media. Lowest growth rate(0.104mm) per hour was observed in Brown's agar medium.

The effect of temperature on mycelial growth of A. alternata revealed that the pathogen could grow in a wide range of temperature 5*C- 40*C(Table 2). The pathogen did not grow at 5*c and 40*c. However optimum temperature for mycelial growth (68.84mm diameter of colony) was found at 30*c followed by 25*c (53.67 mm dia).

The optimum pH for growth of the pathogen was 6.5 at which growth of the pathogen was maximum. Beyond optimum pH, the growth of the pathogen was found decreasing. With the increase of acidity and alkalinity, the growth of the pathogen was adversely affected and was found least at pH2 and 11.5. Maximum degree of spore germination was observed in temperature 25-30*c. The temperature beyond 30*c proved toxic for sporulation. The sporulation started at 10*c and increased gradually with further increase in temperature. No sporulation of the pathogen could be observed at 5*c.

The time required for spore germination was only 6-7 hrs in temperature 20-30*c but was more in 5-15*c and 35-40*c. The time factor plays a much more important role under fluctuating field conditions as regards duration of optimum meteorological situation and subsequent attack on castor. These results were in conformity with Neergaard (1945) and Prasadet al (1973) in different isolates of A.alternata. (Ahmad and Khan (1995), Mishra and Mishra (2012), Sanjeev et al(2017), Narendra Kumar (2017)).

**Table-2**

**Mycelial growth and sporulation of Alternariaalternata at different temperature and pH.**

| Temp (*c) | Dia of Colony (mm) | (vis obv.) Deg of Sporulation | pH. lev | Avg Dia of Colony | (vis obv.) Deg of Sporulation |
|---|---|---|---|---|---|
| **5** | 4.83 | - | 2.0 | 8.66 | - |
| **10** | 11.66 | + | 2.5 | 15.83 | - |
| **15** | 28.83 | ++ | 3.0 | 17.34 | + |
| **20** | 42.16 | +++ | 3.5 | 17.66 | + |
| **25** | 53.67 | ++++ | 4.0 | 20.84 | ++ |
| **30** | 68.84 | ++++ | 4.5 | 24.50 | ++ |
| **35** | 34.83 | ++ | 5.0 | 27.33 | +++ |
| **40** | 5.17 | ++ | 5.5 | 36.00 | ++++ |
| | | | 6.0 | 44.83 | ++++ |
| | | | 6.5 | 59.34 | +++ |
| | | | 7.0 | 44.00 | ++ |
| | | | 7.5 | 43.50 | ++ |
| | | | 8.0 | 36.00 | + |
| | | | 8.5 | 35.33 | + |
| | | | 9.0 | 28.50 | + |
| | | | 9.5 | 28.34 | + |
| | | | 10.0 | 25.16 | + |
| | | | 10.5 | 21.16 | + |
| | | | 11.0 | 11.83 | + |
| | CD at5%P 3.62 | | 11.5 | 7.00 CD at 5%P 8.99 | + |

**Legend-** Nil (-), Poor (+), Moderate (++), Fair (+++), Profuse (++++).

Optimum pH for spore germination of pathogen was found in acidic medium 6.5. Highly acidic or alkaline media were not very favourable for germination. These findings were in agreement with Cochrane (1958) and Webb (1921).

**Conclusion**

Blight disease caused by Alternaria alternata is one of the diseases of castor plant causing heavy losses in yield in India. The investigations were carried out in vitro to determine the effect of culture media, temperature and pH on growth and sporulation on Alternaria alternata. Average mycelial growth rate and sporulation of the pathogen Alternaria alternata was recorded on different synthetic agar culture media. It was found that growth and sporulation of the pathogen was significantly higher on Kirchoff's agar medium. The effect of temperature on mycelial growth and sporulation of the pathogen revealed that optimum temperature for mycelial growth and sporulation was found at 30*c followed by 25*c.The optimum pH for growth and sporulation of the pathogen was found 6.5 at which growth was maximum. The time required for spore germination was recorded 6 -7 hrs. in temperature range 20-30*c but it was more in 5-15*c and 35-40*c.

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